To: Barron, Mace[Barron.Mace@epa.gov]; Holder, Edith[holder.edith@epa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov]
Cc: Peter Meyer[pmeyer@hydrosphere.net]; Cris Griffin[cgriffin@hydrosphere.net]
From: Craig Watts
Sent: Wed 9/21/2016 3:02:23 PM
Subject: RE: Final report for recent round of toxicity tests

Mace,

We are re-running one of the acute studies. The Finasol report will be delayed by a week.

Craig

From: Craig Watts
Sent: Friday, September 16, 2016 9:40 AM
To: 'Barron, Mace' <Barron.Mace@epa.gov>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Mace,

We have made the changes and corrections and renamed the report 16119 REV 091616. Here is the link to the revised report.

https://www.dropbox.com/s/conr0126ep4mepf/16119%20REV%20091616.pdf?dl=0

We have not received any Finquel. Finasol is the one we are wrapping up early next week.

Craig

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: Thursday, September 15, 2016 4:20 PM
To: Craig Watts <craig@hydrosphere.net>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Hey guys:

Just very few minor revisions requested from my technical review:

Table 2: A. Punctulata

* Acute column: please either spell out not applicable in the cell or add a footnote defining "NA".

*chronic column: replace NA with a footnote or something specifying the organism age or life stage tested.

Table 12:

*report the NOEC and IC24 values in uL/L

Please do provide a revised copy, as well as a revised excel sheet with the toxicity summary tables.

Thanks again for your work with EPA and Pegasus.

PS: also, could you update us what is next on your schedule for this work (e.g., finquel? Anything else to be completed from testing samples we have provided?

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Thursday, September 15, 2016 2:35 PM
To: Barron, Mace <Barron.Mace@epa.gov>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Mace,

Spreadsheets? You have a beautiful report in front of you!

Here is your spreadsheet.

Craig

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: Thursday, September 15, 2016 2:56 PM
To: Craig Watts <craig@hydrosphere.net>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Thank you!

I was able to download a copy and will provide a technical review in next few days.

Could you also provide a copy of just the tox results in excel format similar to what you provided for the dilbits (attached).

Much appreciated,

Mace

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Thursday, September 15, 2016 1:49 PM
To: Barron, Mace <Barron.Mace@epa.gov>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: Final report for recent round of toxicity tests

To all,

So much for our effort to simply and streamline the reports. The Corexit report weighs in at over 15 MB and 111 pages. Instead of choking everyone's email server, I will share a link to the file on our DropBox account:

https://www.dropbox.com/s/aoi238renwts50v/16119.pdf?dl=0

Please look over the report and let us know if you have any questions or if you would like to see any changes.

We have all of the testing completed for the Finasol product with the exception of the two acute EC50 tests; they are going up today. The report for Finasol should go out this same time next week.

Regards,

Craig

Providing Environmental & Product Toxicity Testing since 1986

Craig Watts, Lab Director

Hydrosphere Research

11842 Research Circle

Alachua, FL 32615-6817

T (386) 462-7889

www.hydrosphere.net

***Supporting Information**

Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500

Mobing Zhuang[1], Gulizhaer Abulikemu[2], Pablo Campo[3]*, William Platten III[4], Makram T. Suidan[5], Albert D. Venosa (retired)[4]and Robyn N. Conmy[4]

1 Department of Biomedical, Chemical and Environmental Engineering, University of Cincinnati, 2901 Woodside Drive, Cincinnati, OH 45221, USA

2 Pegasus Technical Services Inc., 46 E Hollister St, Cincinnati, OH 45219 USA

3 Cranfield Water Science Institute, Cranfield University, Cranfield, Beds, MK43 0AL, UK

4 U.S. Environmental Protection Agency, NRMRL, 26 W. MLK Drive Cincinnati, OH, 45268, USA

5 Faculty of Engineering and Architecture, American University of Beirut, Bechtel Engineering Bldg. - 3rd flr. - Room 308 P.O. Box: 11-0236 Riad El Solh 1107 2020, Beirut, Lebanon

*Corresponding author: Pablo Campo
Tel: +44 (0)1234 754332
E-mail address: p.campo-moreno@cranfield.ac.uk

Table S1. Summary of Experimental Layout for High Concentration Experiment.

Test	Temperature	Treatment	Sampling Events	Sample Replicates	Total Experimental Units (EU)
1	5 °C	C9500 alone	11	3	33
2	5 °C	ANS dispersed by C9500	11	3	33
3	5 °C	alone	11	3	33
4	5 °C	Killed ANS control	1	3	3
5	5 °C	Killed C9500 control	11	3	33
6	5 °C	Killed ANS+C9500 control	11	3	33
				Subtotal EU's	**168**
7	25 °C	C9500 alone	9	3	27
8	25 °C	ANS dispersed by C9500	9	3	27
9	25 °C	ANS alone	9	3	27
10	25 °C	Killed ANS control	1	3	3
11	25 °C	Killed C9500 control	9	3	27
12	25 °C	Killed ANS+ C9500 control	9	3	27
				Subtotal EU's	**138**
		Total EU's for High Concentration Experiment			**306**

Table S2. Summary of Experimental Layout for Low Concentration Experiment.

Test	Temperature	Treatment	Sampling Events	Sample Replicates	Total Experimental Units (EU)
13	5 °C	C9500 alone	11	3	33
14	5 °C	ANS+C9500	11	3	33
15	5 °C	ANS alone	11	3	33
16	5 °C	Killed ANS control	1	3	3
17	5 °C	Killed C9500 control	11	3	33
18	5 °C	Killed (ANS+C9500) control	11	3	33
19	5 °C	Killed (ANS+C9500) +Protease	6	3	18
				Subtotal EU's	**186**
20	25 °C	C9500 alone	10	3	30
21	25 °C	ANS+C9500	10	3	30
22	25 °C	ANS alone	10	3	30
23	25 °C	Killed ANS control	1	3	3
24	25 °C	Killed C9500 control	10	3	30
25	25 °C	Killed (ANS+C9500) control	10	3	30
26	25 °C	Killed (ANS+C9500) +Protease	6	3	18
				Subtotal EU's	**181**
		Total EU's for Low Concentration Experiment			**357**

Table S3. First-order biodegradation rate coefficients and standard deviations (sd) of individual alkanes in the high concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
C10	0.19	0.02	1.34	0.18	0.63	0.03	2.00	0.34
C11	0.15	0.01	1.16	0.15	0.40	0.02	1.30	0.09
C12	0.12	0.01	1.02	0.14	0.29	0.03	0.96	0.06
C13	0.11	0.01	0.94	0.14	0.27	0.03	0.91	0.05
C14	0.11	0.01	0.92	0.14	0.25	0.03	0.82	0.04
C15	0.11	0.01	0.95	0.14	0.24	0.03	0.84	0.05
C16	0.11	0.01	1.01	0.15	0.24	0.03	0.92	0.05
C17	0.11	0.01	1.08	0.16	0.24	0.03	0.97	0.06
PR	0.15	0.03	0.45	0.11	0.07	0.01	0.39	0.04
C18	0.11	0.01	1.06	0.14	0.22	0.02	0.96	0.05
PH	0.15	0.03	0.46	0.12	0.08	0.01	0.43	0.04
C19	0.11	0.01	1.05	0.14	0.22	0.02	0.97	0.06
C20	0.11	0.01	0.99	0.14	0.21	0.02	0.93	0.05
C21	0.10	0.01	0.97	0.14	0.20	0.02	0.91	0.05
C22	0.10	0.01	0.88	0.13	0.19	0.02	0.84	0.04
C23	0.09	0.01	0.84	0.13	0.18	0.02	0.79	0.04
C24	0.09	0.01	0.84	0.14	0.17	0.02	0.79	0.04
C25	0.09	0.01	0.81	0.13	0.15	0.02	0.81	0.03
C26	0.08	0.01	0.81	0.14	0.12	0.02	0.78	0.04
C27	0.08	0.01	0.74	0.13	0.11	0.01	0.71	0.05
C28	0.12	0.02	0.75	0.12	0.12	0.02	0.60	0.09
C29	0.12	0.02	0.81	0.15	0.12	0.02	0.81	0.07
C30	0.10	0.01	0.83	0.14	0.12	0.02	0.74	0.04
C31	0.09	0.01	0.81	0.14	0.09	0.01	0.61	0.05
C32	0.10	0.01	0.81	0.14	0.09	0.01	0.60	0.06
C33	0.09	0.01	0.68	0.09	0.07	0.01	0.56	0.06
C34	0.08	0.01	0.60	0.09	0.05	0.01	0.54	0.05
C35	0.07	0.01	0.53	0.09	0.04	0.01	0.58	0.04

Table S4. First-order biodegradation rate coefficients and standard deviations (sd) of individual alkanes in low concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
C10	0.47	0.03	0.93	0.16	0.63	0.04	NA1	NA1
C11	0.25	0.02	0.57	0.07	0.25	0.03	0.47	0.02
C12	0.20	0.01	0.38	0.06	0.23	0.02	0.24	0.03
C13	0.18	0.01	0.42	0.06	0.21	0.02	NA2	NA2
C14	0.13	0.01	0.28	0.04	0.15	0.01	NA2	NA2
C15	0.14	0.01	0.35	0.04	0.15	0.01	NA2	NA2
C16	0.14	0.01	0.37	0.04	0.15	0.02	NA2	NA2
C17	0.13	0.01	0.36	0.04	NA2	NA2	NA2	NA2
PR	NA2	NA2	0.04	0.01	NA2	NA2	NA2	NA2
C18	NA2	NA2	0.35	0.04	NA2	NA2	NA2	NA2
PH	NA2	NA2	0.03	0.01	NA2	NA2	NA2	NA2
C19	NA2	NA2	0.32	0.04	NA2	NA2	NA2	NA2
C20	NA2	NA2	0.30	0.04	NA2	NA2	NA2	NA2
C21	NA2	NA2	0.29	0.04	NA2	NA2	NA2	NA2
C22	NA2	NA2	0.17	0.03	NA2	NA2	NA2	NA2
C23	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C24	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C25	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C26	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C27	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C28	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C29	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C30	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C31	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C32	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C33	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C34	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2
C35	NA2	NA2	NA2	NA2	NA2	NA2	NA2	NA2

NA1: Rate not calculated because of excessively rapid removal after acclimation.
NA2: Rate not calculated because noted compounds were persisted during the experiment.

Table S5. First-order degradation rate coefficients and standard deviations (sd) of individual PAHs in high concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
nap	0.44	0.04	0.68	0.05	0.71	0.02	1.94	0.02
C1-nap	0.23	0.02	0.60	0.04	0.41	0.03	1.84	0.04
C2-nap	0.17	0.02	0.38	0.07	0.26	0.02	0.61	0.03
C3-nap	0.27	0.02	0.35	0.10	0.20	0.02	0.40	0.05
C4-nap	0.13	0.02	0.29	0.10	0.16	0.02	0.35	0.05
phe	0.19	0.01	0.37	0.09	0.26	0.02	0.46	0.05
C1-phe	0.12	0.01	0.34	0.12	0.19	0.02	0.37	0.06
C2-phe	0.05	0.01	0.21	0.07	0.19	0.03	0.27	0.04
C3-phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
flu	0.19	0.02	0.37	0.10	0.26	0.02	0.44	0.05
C1-flu	0.15	0.02	0.35	0.11	0.21	0.02	0.39	0.06
C2-flu	0.05	0.01	0.20	0.07	0.11	0.01	0.27	0.04
C3-flu	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
dbt	0.25	0.01	0.43	0.08	0.44	0.02	0.77	0.03
C1-dbt	0.13	0.01	0.32	0.10	0.22	0.02	0.41	0.04
C2-dbt	0.04	0.01	0.17	0.06	0.11	0.01	0.26	0.04
C3-dbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pyr*	--	--	--	--	--	--	--	--
C1-pyr	0.00	0.00	0.00	NA	NA	0.00	NA	NA
C2-pyr	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

NA: Rate not calculated because noted compounds were persisted during the experiment.
* Not detected in any samples.

Table S6. First-order degradation rate coefficients and standard deviations (sd) of individual PAHs in low concentration experiment.

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+C9500		25 °C ANS+C9500	
	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})	rate (d^{-1})	sd (d^{-1})
nap	NA	NA	0.29	0.05	0.43	0.09	0.36	0.03
C1-nap	NA	NA	0.24	0.03	0.44	0.09	0.28	0.03
C2-nap	NA	NA	0.17	0.04	0.40	0.09	0.21	0.04
C3-nap	NA	NA	0.18	0.04	0.31	0.06	0.20	0.04
C4-nap	NA	NA	0.13	0.03	0.15	0.02	0.14	0.02
phe	NA	NA	NA	NA	NA	NA	0.19	0.03
C1-phe	NA	NA	NA	NA	0.18	0.03	0.20	0.03
C2-phe	0.25	0.01	0.14	0.03	0.11	0.01	NA	NA
C3-phe	0.02	0.01	0.02	0.00	0.00	0.00	0.00	0.00
C4-phe	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
flu	NA	NA	NA	NA	0.29	0.06	NA	NA
C1-flu	NA	NA	NA	NA	0.15	0.02	0.00	0.00
C2-flu	0.29	0.03	0.14	0.03	0.21	0.01	0.00	0.00
C3-flu	0.02	0.00	0.03	0.00	0.00	0.00	0.02	0.00
dbt	NA	NA	0.00	0.00	0.31	0.11	0.00	0.00
C1-dbt	NA	NA	NA	NA	0.23	0.05	0.29	0.06
C2-dbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-dbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
nbt	0.00	0.00	NA	NA	0.00	0.00	0.31	0.11
C1-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-nbt	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
pyr*	--	--	--	--	--	--	--	--
C1-pyr	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.07
C2-pyr	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C1-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C2-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C3-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
C4-cry	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

NA: Rate not calculated because of excessively rapid removal after acclimation.
*Not detected in any samples.

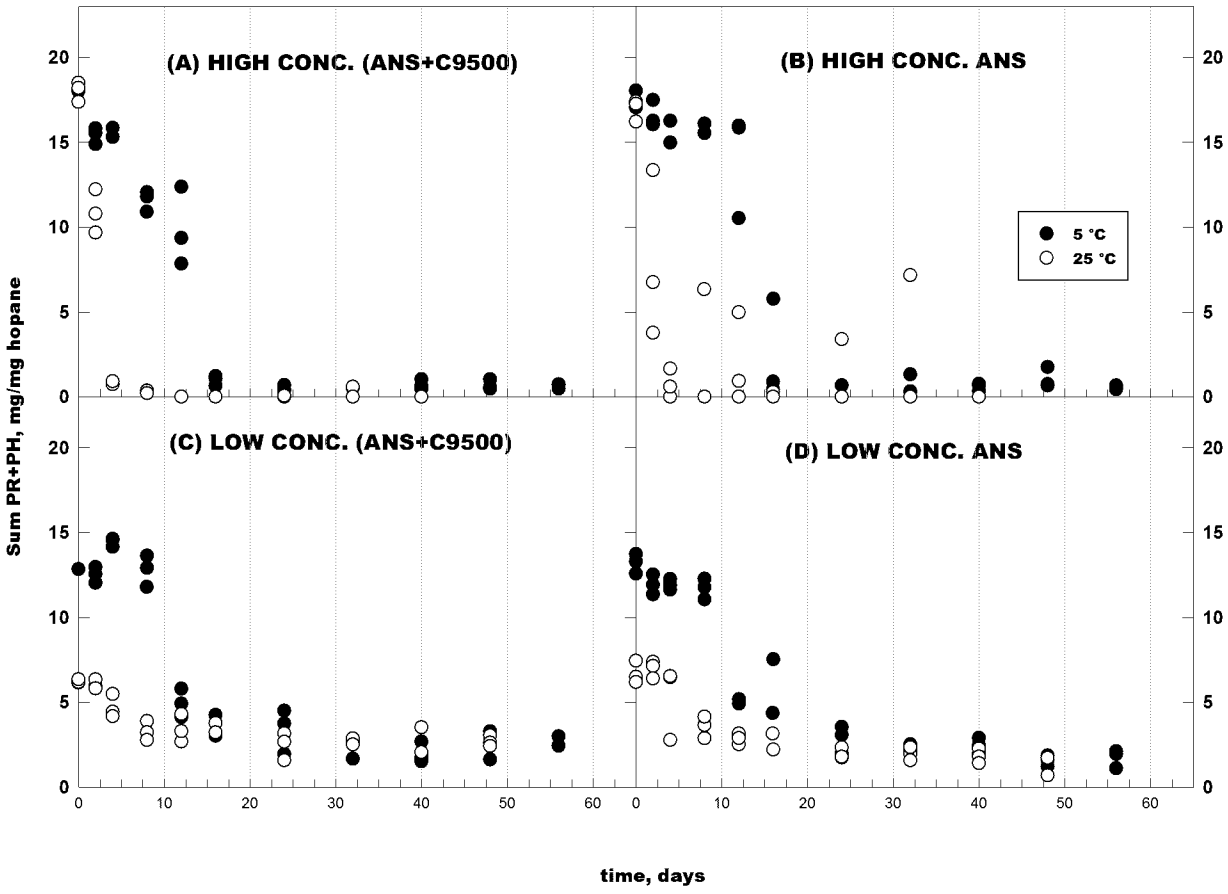

Figure S1. Biodegradation of branched alkanes in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

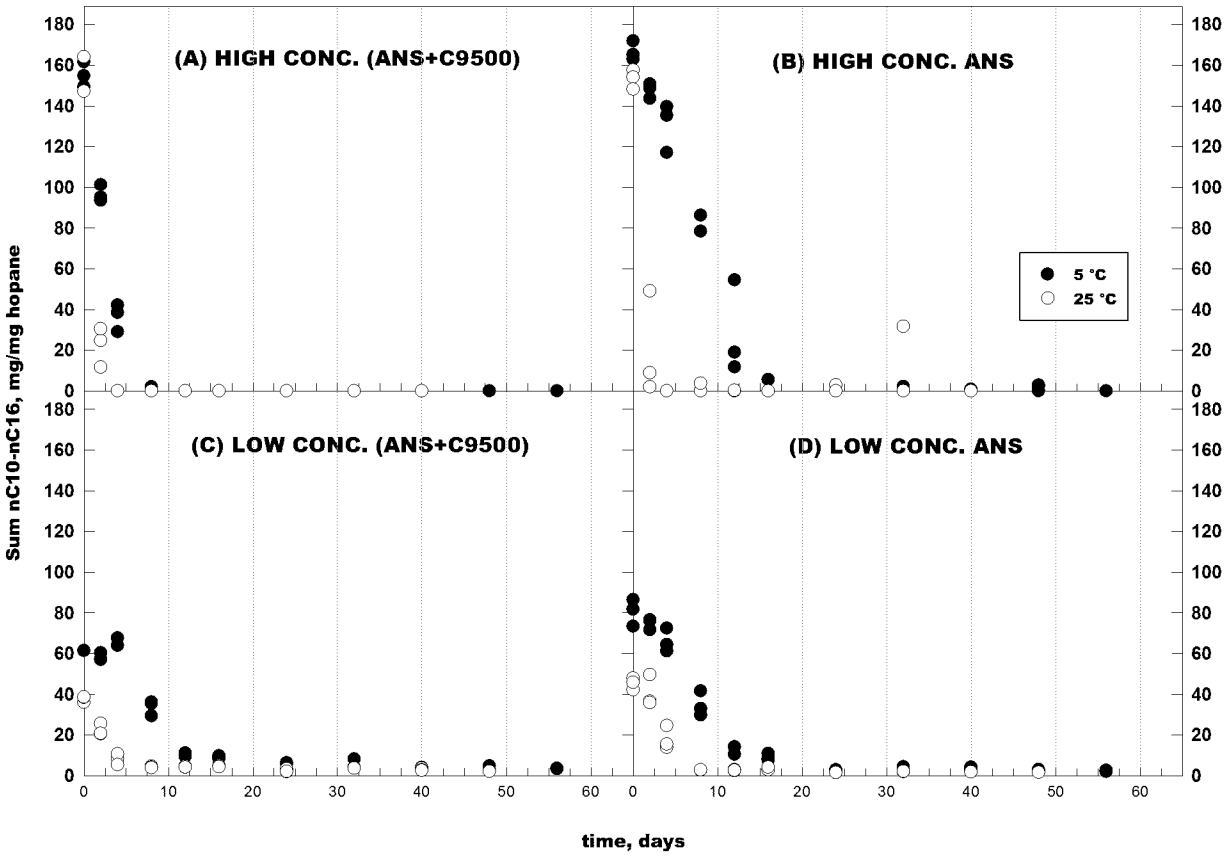

Figure S2. Biodegradation of n-alkanes (nC10-nC16) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

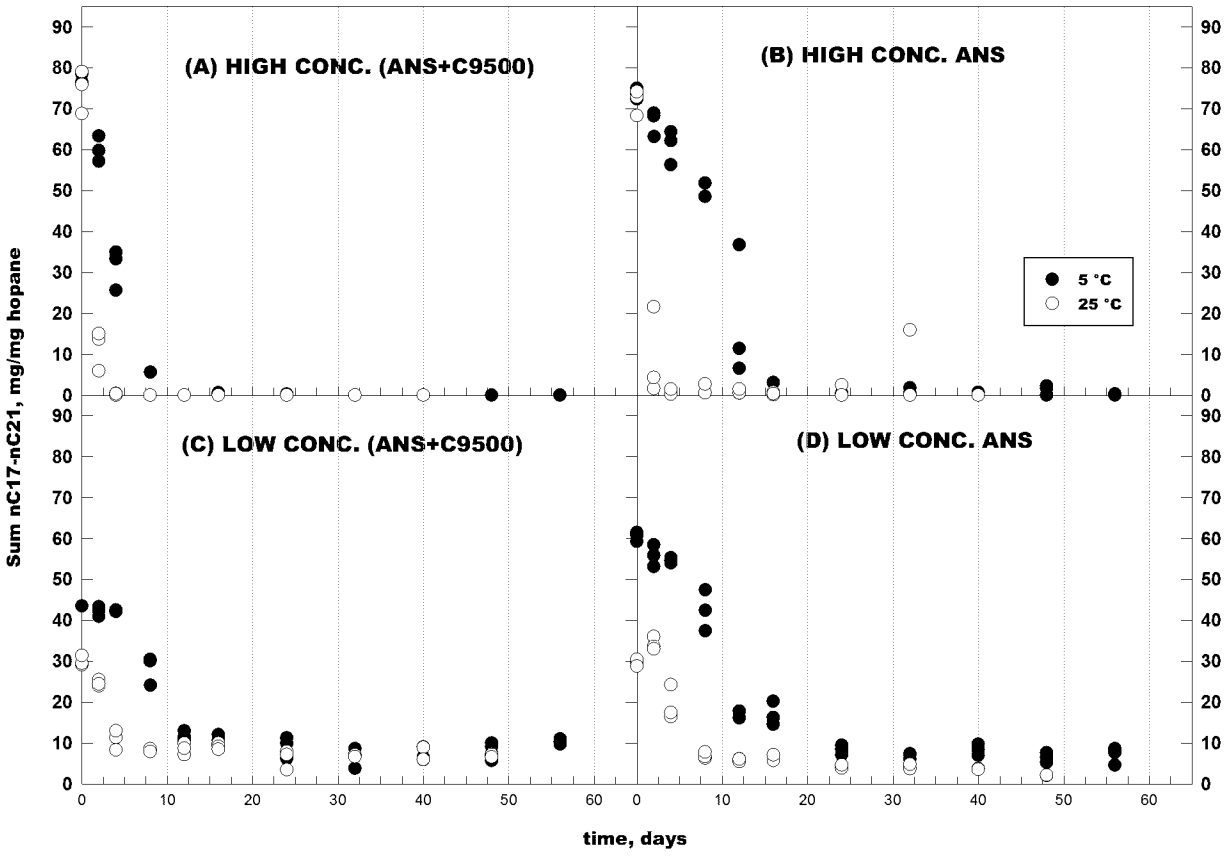

Figure S3. Biodegradation of n-alkanes (nC17-nC21) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

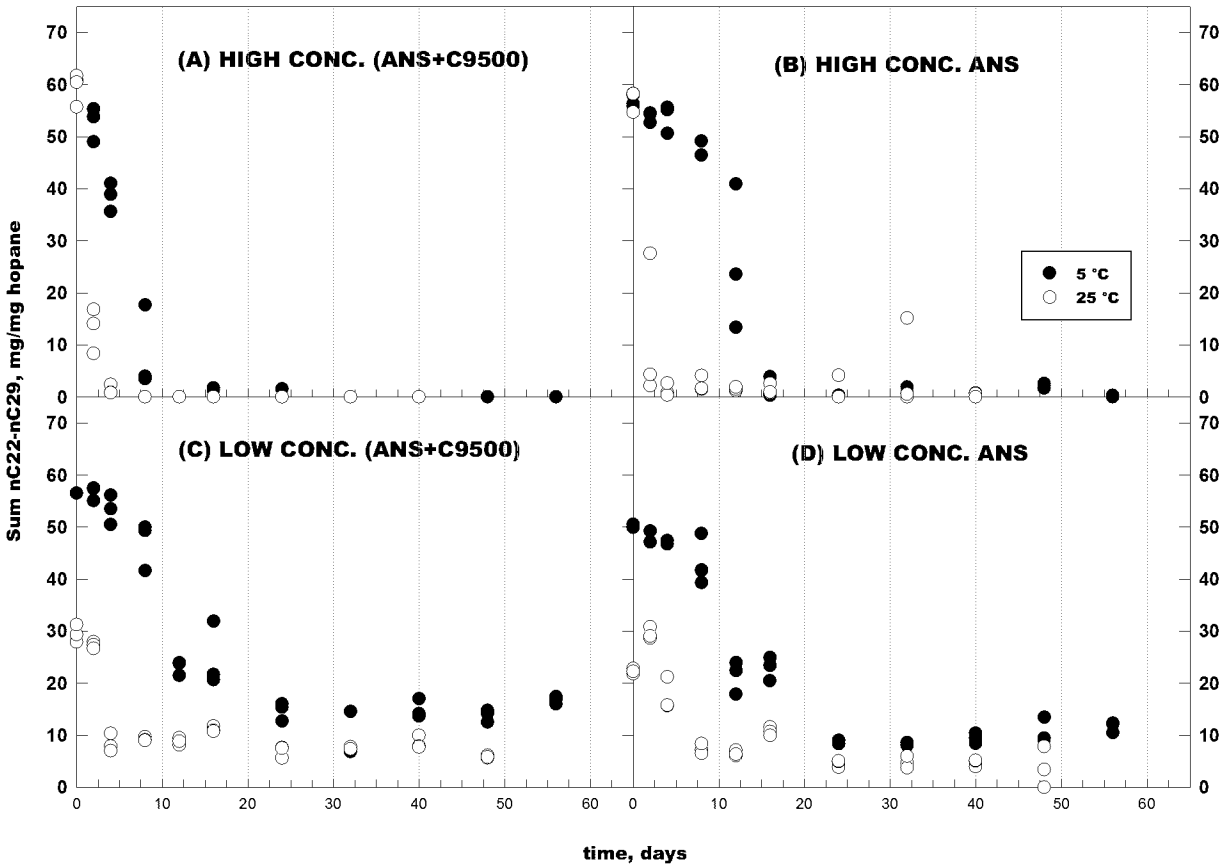

Figure S4. Biodegradation of n-alkanes (nC22-nC29) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

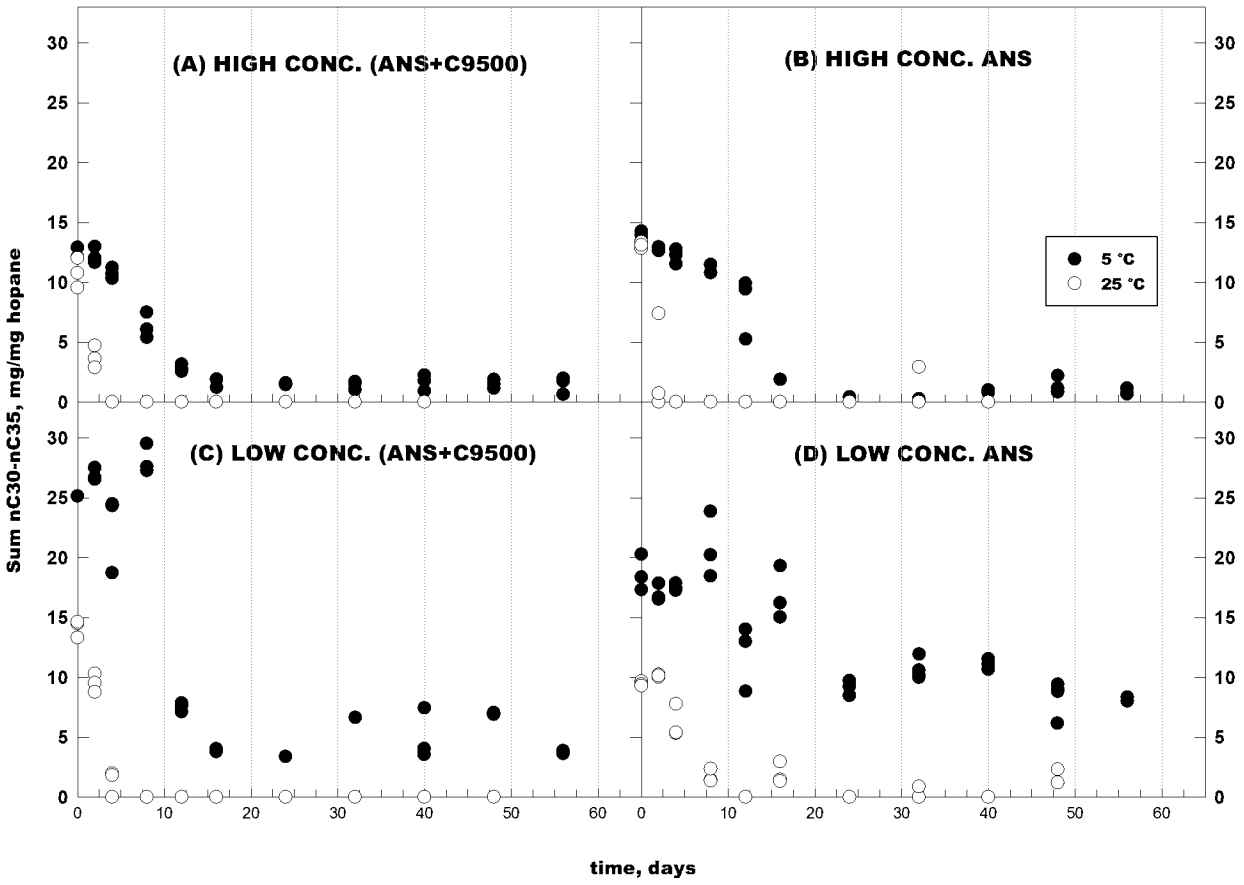

Figure S5. Biodegradation of n-alkanes (nC30-nC35) in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

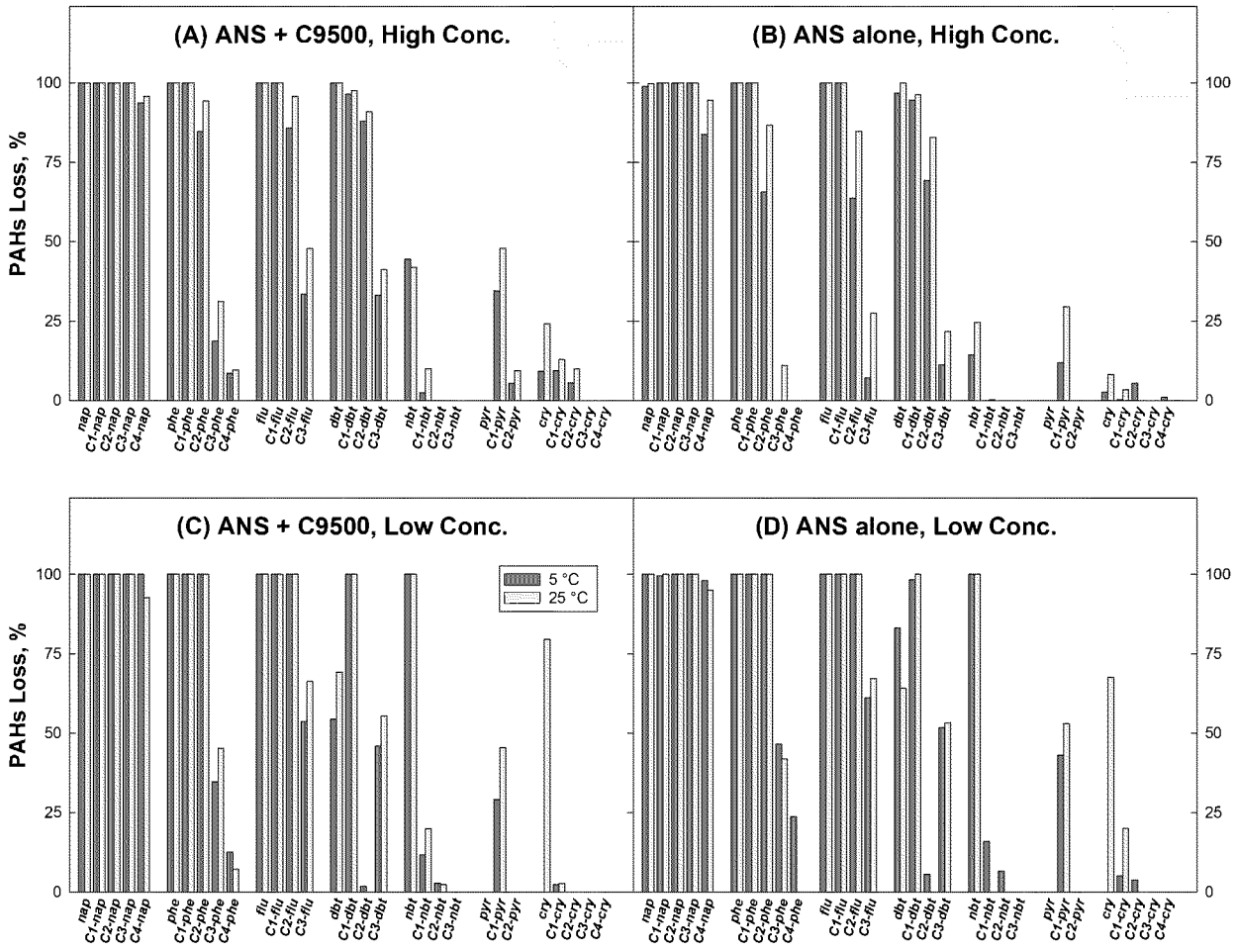

Figure S6. Average loss percentage of individual PAH in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C in high concentration (A, B) and low concentration (C, D) experiment.

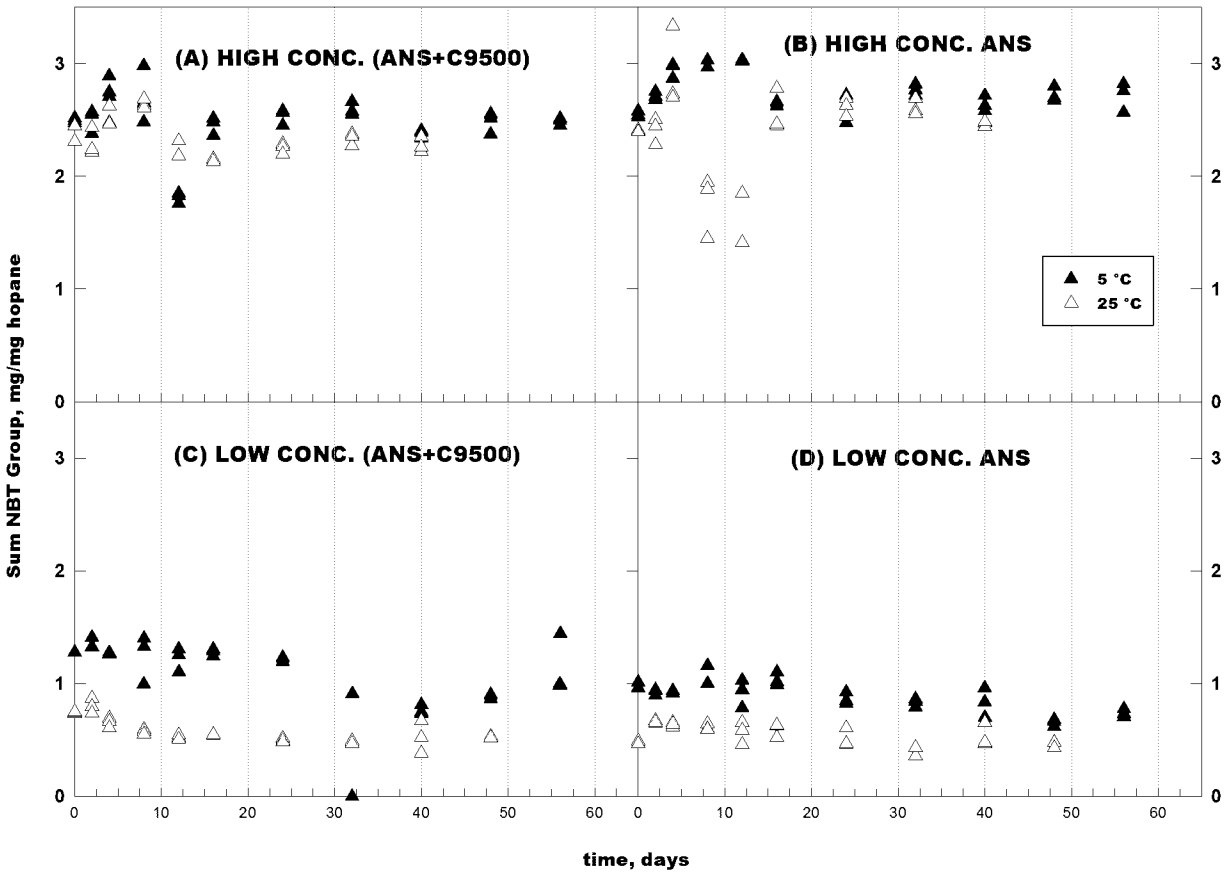

Figure S7. Biodegradation of naphthobenzothiophene homologues in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

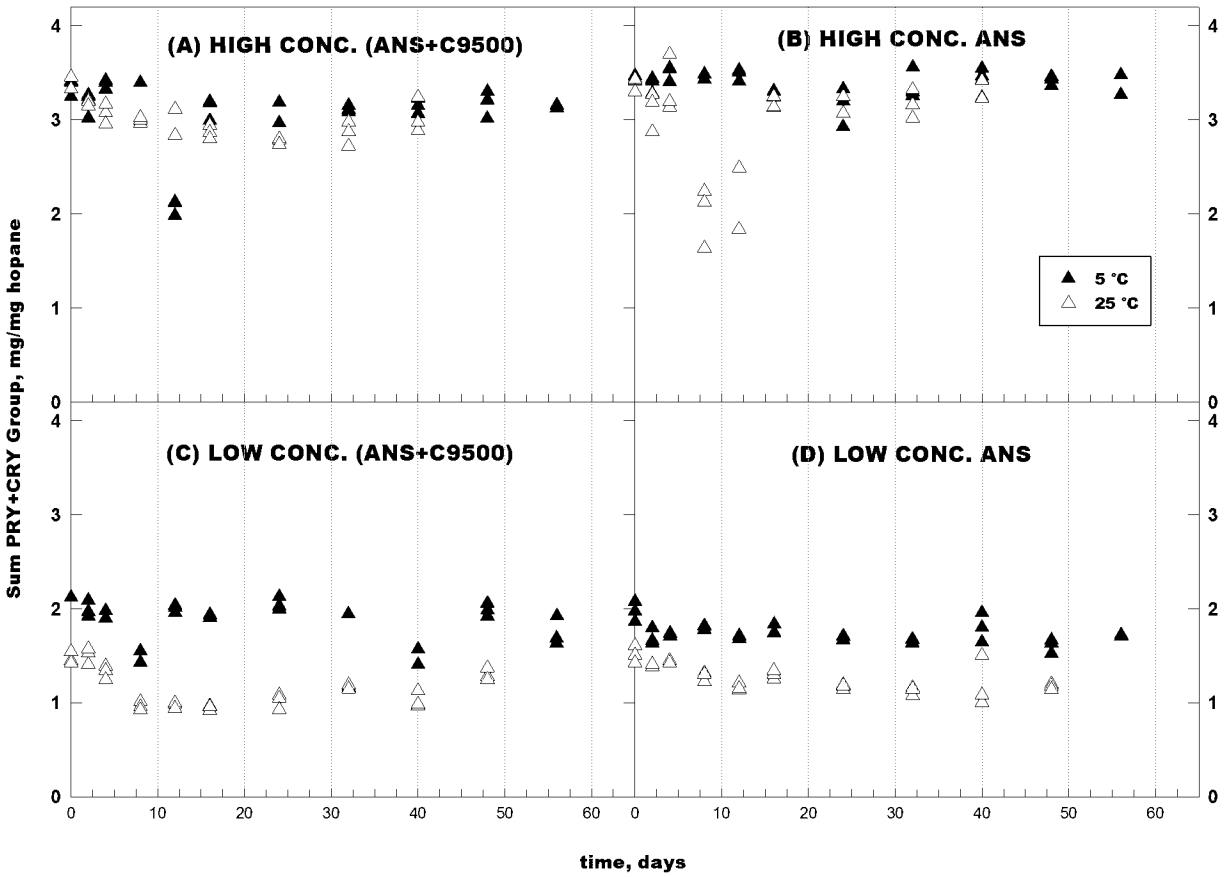

Figure S8. Biodegradation of pyrene homologues + chrysene homologues in the presence (A, C) and absence (B, D) of C9500 at 5 °C and 25 °C. High concentration treatments are in panel A and B, whereas low concentration treatments are in panels C and D.

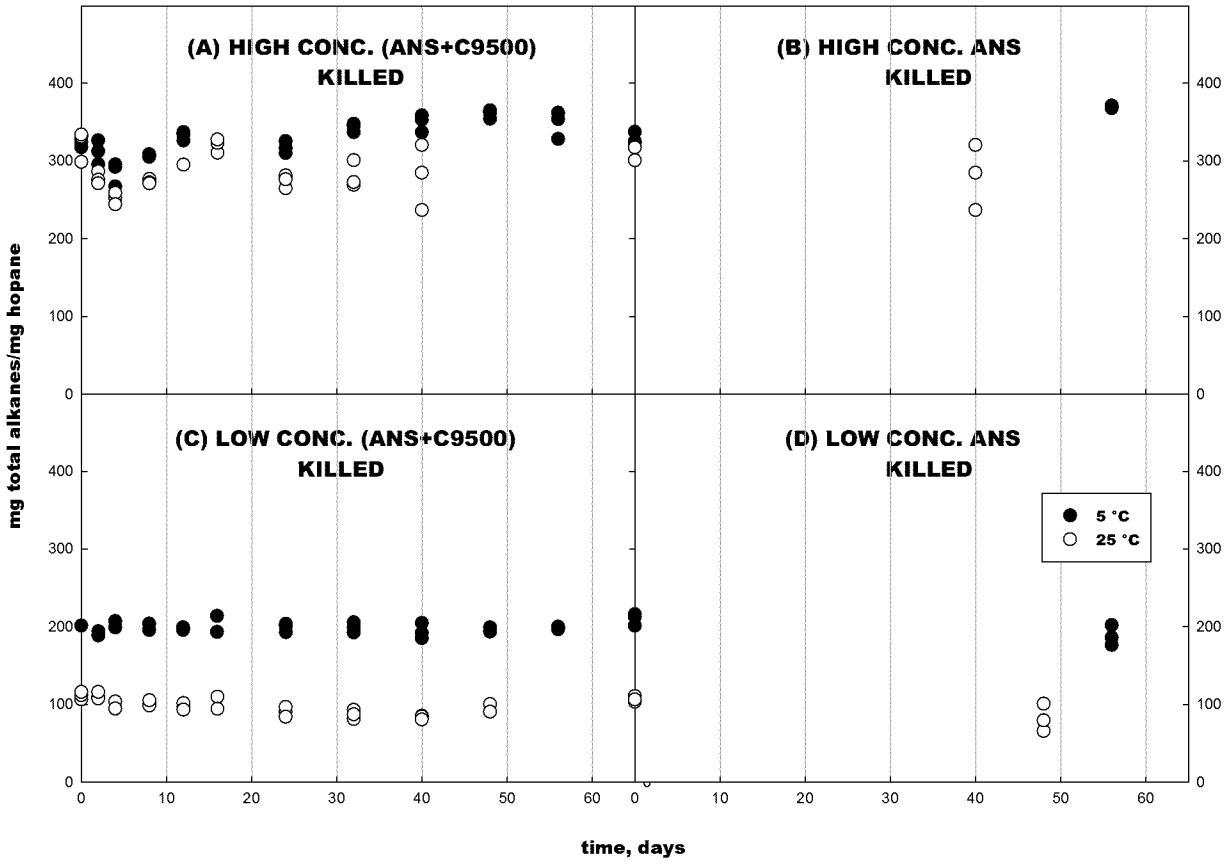

Figure S9. Biodegradation of hopane-normalized total alkanes at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D) in killed control samples.

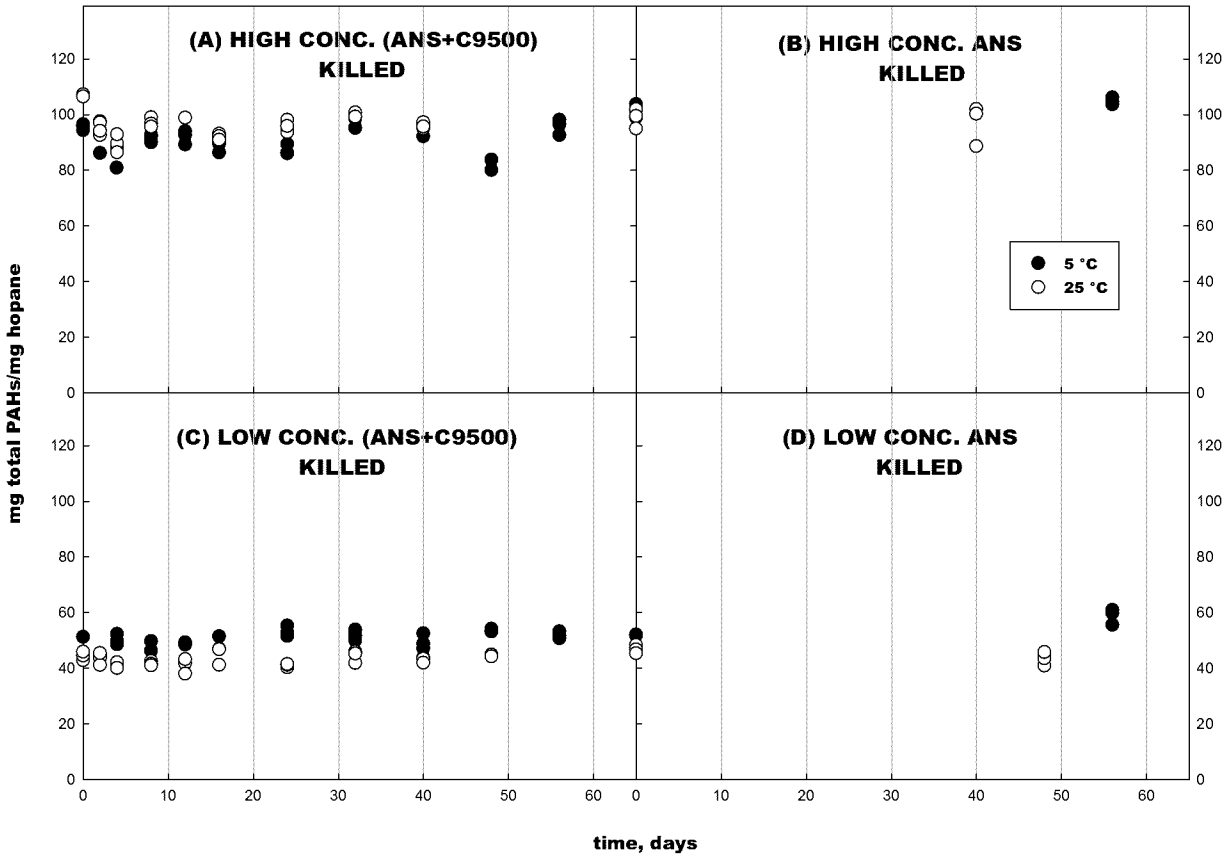

Figure S10. Biodegradation of hopane-normalized total PAHs at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D) in killed control samples.

Biodegradability of Different Initial Concentrations of Alaska North Slope Crude Oil Dispersed with Corexit C9500

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ABSTRACT

In-situ concentrations of dispersant and crude oil observed during the Deepwater Horizon oil spill were lower than typically studied. To better understand biodegradation of the low oil and dispersant concentrations observed, laboratory experiments were conducted with Alaska North Slope crude oil (ANS) and the dispersant Corexit 9500 (C9500) to evaluate the removal of selected chemicals including: dioctyl sodium succinate (DOSS) which is an anionic surfactant in C9500, alkanes, and polycyclic aromatics hydrocarbons (PAHs). The study considered the biodegradability of ANS alone, ANS dispersed with C9500 at a dispersant-to-oil ratio of 1:25, and C9500 alone. Oil loads of 1,000 and 40 μL/L were compared at two temperatures with microbial cultures acclimated to the temperature 25 (meso culture) and 5 (cryo culture) °C. The biodegradation rate of DOSS, by the meso culture (25 °C) was approximately an order of magnitude faster in the high concentration experiment than in the low concentration experiment. At the lower temperature, the deep-water culture cryo (5 °C) had limited ability to metabolize DOSS regardless of the initial concentrations. Biodegradation of oil components was favored by the presence of dispersant as C9500 shortened lag phases and enhanced biodegradation rates. Alkanes and PAHs were degraded more rapidly in the high concentration samples. In the low concentration experiment, the percentage of alkanes persisting was higher than in the high concentration experiment (8-18% vs below 1%). No significant lag period in PAH biodegradation was observed in the high oil concentration experiment compared to the low oil concentration experiment where a lag period of 12 to 16 d was observed. The extent of biodegradation of some of the less soluble aromatic compounds increased by 4-86% under low oil concentration conditions.

KEYWORDS

Corexit 9500, biodegradation, initial oil concentration

INTRODUCTION

To minimize the impact of oil spills, responders adopt the Net Environmental Benefit Analysis, this is, any response technique should decrease the environmental costs rather than increase them (API, 2013). When mechanical recovery or in situ burning cannot achieve the goal of protecting shorelines from oil, the impact of using dispersants is likely favored by the benefits, which include keeping oil from sensitive areas and speeding up biodegradation by breaking an oil slick into small droplets (Prince, 2015). Thus, it is important to study the biodegradability of dispersants and dispersed oil under various conditions for better informed use in future applications.

In the aftermath of the Deepwater Horizon blowout in the Gulf of Mexico (GOM), comprehensive monitoring of the dispersant Corexit 9500 (C9500) and hydrocarbons in both surface and subsurface environments took place. In samples collected near the Macondo well, concentrations of dioctyl sodium sulfosuccinate (DOSS), an anionic surfactant present in C9500, ranged from 0.4 to 12 μg/L (Kujawinski et al., 2011). After the spill, DOSS was detected in subsurface waters at concentrations below 40 μg/L at different locations in the GOM, while in one surface water sample close to the wellhead concentration exceeded 200 μg/L (Gray et al., 2014). The combination of turbulence at the GOM surface and the dispersant application could rapidly decrease oil concentrations to below 100 mg/L, dropping even further over time (Lee et al., 2013). Hence, these researchers recommended that biodegradation tests of dispersed oil should be conducted under more dilute concentrations to mimic real conditions.

Most studies published on biodegradation of oil and dispersants prior to the Deepwater incident had been conducted at concentrations well-above the reported in situ levels (Operational

Science Advisory Team, 2010; Prince, 2015). Several studies assessing biodegradation of oil dispersed by C9500 used initial oil and dispersant concentrations from 100 to 4,500 mg/L, with dispersant-to-oil ratios (DOR) of 1:10, 1:20, or 1:25 (Campo et al., 2013; Lindstrom and Braddock, 2002; Zahed et al., 2010). A few studies were published on oil biodegradation with initial oil and dispersant concentrations below 100 μL/L (Brakstad et al., 2015; Prince et al., 2013; Venosa and Holder, 2007).

Our objective was to determine how the initial amounts of oil and dispersant affect their biodegradation. Thus, we conducted high (1000 μL/L) and low oil concentration (40 μL/L) biodegradation experiments with Alaska North Slope crude oil (ANS) and C9500 at 5 and 25 °C. C9500 is a dispersant included in the National Contingency Plan Product Schedule and was applied to both the surface and submarine environment in the GOM (Operational Science Advisory Team, 2010). Performance, evaluated by measuring time-varying DOSS, and oil chemicals in high and low concentration experiments at 5 and 25 °C are reported in this paper.

MATERIALS AND METHODS

Chemicals and Reagents

The U.S. Environmental Protection Agency (EPA) provided the ANS and the dispersant C9500 (Nalco Naperville, IL) used in this study. Standards for DOSS and its deuterated surrogate (D_{17}-DOSS) were obtained from Aldrich (St. Louis, MO) and Isotec (Miamisburg, OH), respectively. Acetonitrile, deionized ultra-filtered water, and mineral salts were purchased from Fisher Scientific (Pittsburgh, PA). Dichloromethane (DCM) was obtained from Tedia (Fairfield, OH). Sylon CT (Sigma-Aldrich, St. Louis, MO) was used for the deactivation of the glassware to prevent adherence of oil and biomass to the sides of the flasks. The protease

cocktail for inhibiting enzymatic activity was also acquired from Sigma-Aldrich (St. Louis, MO).

Protease Solution Preparation

Following the instruction provided with the protease cocktail, 5 mL dimethyl sulfoxide (DMSO) was added to 1,075 mg lyophilized powder, which was previously stored unopened at 20 °C. The solution was vortexed for ten minutes before the addition of 20 mL deionized water. The resulting solution was clear. The reconstituted protease solution contained the following inhibitors: 4-(2-aminoethyl) benzenesulfonyl fluoride hydrochloride (23 mM), ethylenediaminetetraacetic acid (100 mM), bestatin (2 mM), pepstatin A (0.3 mM), and E-64 (0.3 mM).

Artificial Seawater

The salts in the GP2 medium, dissolved in DI water, were (expressed in g/L) NaCl (21.03), $MgCl_2 \cdot 6H_2O$ (9.5), Na_2SO_4 (3.52), $CaCl_2 \cdot 2H_2O$ (1.32), KCl (0.61), KBr (0.088), $NaB_4O_7 \cdot 10H_2O$ (0.034), $SrCl_2 \cdot 6H_2O$ (0.02), $NaHCO_3$ (0.17) $FeCl_3 \cdot 6H_2O$ (0.05), $Na_5P_3O_{10}$ (0.297) and KNO_3 (2.89). The GP2 pH was ≈ 7.5. The GP2 medium was autoclaved at 121 °C for 30 min and then used as the sterile matrix in this study (Spotte et al., 1984).

Microbial Cultures

The meso culture was originally isolated from the surface water of the GOM in the vicinity of the Macondo well. The cryo culture was isolated from water close to the plume location at a depth of 1,240 m near the wellhead . Both cultures were isolated by EPA's Gulf Breeze, FL research laboratory. Enriched inocula were prepared as follows: in 2-L shake flasks, 2.5 mL South Louisiana crude oil (SLC) were added to 500 mL of the original unfiltered GOM

seawater supplemented with 2.8 g/L KNO_3, 0.55 g/L NaP_3O_{10}. The flasks were rotated (400 rpm) at 5 °C (cryo culture) and 25 °C (meso culture). Thegrowth times were 7 d for the meso culture and 17 d for the cryo culture. After these periods, cells were harvested by centrifugation (6,000 × g for 30 min at 4 °C) and the pellets preserved in 10% glycerol at -70 °C. Before shipping to EPA-Cincinnati, 1 mL of each thawed culture were added to shake flasks containing 500 mL GP2 medium fortified with 2.5 mL of SLC. The cultures used in this study were grown at EPA (Cincinnati, OH) by transferring the original cultures from EPA's Gulf Breeze to GP2 medium supplemented with SLC. The meso culture was harvested after 3 weeks of growth and the cryo culture after 5 weeks. The harvested culture was processed in the same fashion as mentioned previously: centrifugation (6,000 × g for 30 min at 4 °C) and the pellets preserved in 10% glycerol at -70 °C. For use in experiments, the cultures were defrosted at room temperature, washed with 0.85% saline solution to remove glycerol, centrifuged, and brought back up to the frozen volume.

Experimental Design

A summary of the experimental design is shown in Tables S1 and S2 for the high and low oil concentration experiments, respectively. For the high concentration experiments, 6 treatments were tested at 5 and 25 °C in triplicate: C9500 dispersant alone, ANS oil alone, ANS dispersed with C9500, and the corresponding killed controls (KCs). The 5 °C experiment required 11 sampling events in triplicate (0, 2, 4, 8, 12, 16, 24, 32, 40, 48 and 56 d), while 9 events were used for the 25 °C (0, 2, 4, 8, 12, 16, 24, 32 and 40 d). At each sampling event, triplicate shake flasks of each treatment along with KCs were sacrificed, except for the ANS alone KCs which were analyzed at the beginning and end of the experiment.

The same treatments were included in the low concentration experiment, but both oil and dispersant concentrations were prepared at 4% of the initial loads used in the high concentration experiment. Additionally, a seventh treatment involved the use of triplicate KCs containing the enzyme protease (0.5 mL) to neutralize the enzymatic DOSS hydrolysis. This treatment was included only in the low concentration experiment. The sampling events were conducted similarly to the high concentration experiment with the exception of the protease treatment, which was sampled at 0, 12, 24, 35, 46 and 56 d and 0, 8, 18, 28, 38 and 48 d for the 5 and 25 °C experiments, respectively.

After all treatments were prepared, the appropriate cultures were spiked (0.5 mL) and the flasks were placed on orbital shakers. The shakers were operated at 200 rpm and kept in the appropriate constant temperature rooms until the expected sampling events. All KCs were sterilized by adding 1 mL sodium azide stock (50 g/L) into the 100 mL of GP2 medium.

High Concentration Treatments

To evaluate the biodegradation of dispersed oil, 2 L baffled beakers were used in which 40 µL of C9500 and 1,000 µL of ANS were added to 1,200 mL of GP2 to achieve a volumetric DOR of 1:25. Subsequently, the beaker containing the mixture was shaken at 200 rpm for 10 minutes and then left stationary for an additional 10 min, then the dispersion was transferred to a 20-L continuously mixed carboy. The stationary time was to allow undispersed oil to rise to the top layer of water and draining the dispersion into carboy should be carefully done (not disturbing the top layer of undispersed oil). The above procedure was repeated until the volume in the carboy reached 14 L. Subsequently, 100 mL aliquots of the mixtures were transferred to the shake flasks.

The second treatment was for the evaluation of oil as the only substrate. ANS (100 µL) was added directly to shake flasks containing 100 mL sterile GP2 medium, which yielded an estimated oil concentration of 1,000 µL/L. To evaluate the degradation of dispersant alone, 480 µL of C9500 was added to 14 L of sterile GP2 medium in a continuously mixed carboy; after 30 min, 100 mL aliquots of this mixture were dispensed into shake flasks for this treatment.

Low Concentration Treatments

To prepare the low concentration dispersed oil, a single batch of 1,200 mL GP2 was spiked with 200 µL ANS onto the water surface followed by addition of 8 µL C9500 onto the oil slick, which yielded a volumetric DOR of 1:25 in a 2 L baffled beaker. The beaker was placed on a shaker and agitated for 20 minutes. The shaker was started at a very slow speed and was ramped up to 200 rpm gradually. After mixing, the beaker remained stationary for 10 minutes and, subsequently, the dispersion was carefully drained into a 20 L continuously mixing carboy. The above procedure was repeated until the volume in the carboy reached 3 L, and then 12 L of sterile GP2 was added to dilute the mixture. The shaker was started at a very slow speed and was ramped up to 200 rpm gradually. The beaker was placed on a shaker for 20 min and then remained stationary for 10 minutes. Subsequently, 3 L of the dispersion were poured into a 20 L carboy under continuous mixing and 12 L of sterile GP2 was added for dilution purposes. Overall, the oil and dispersant amounts in this treatment were approximately 4% of that in the high concentration experiment. Finally, shake flasks were filled with 100 mL aliquots of the diluted dispersed oil. Low concentration ANS alone was prepared by spiking 4 µL of oil into shake flasks containing 100 mL sterile GP2, which yields an oil concentration of 40 µL/L (i.e., 4% of the high concentration oil alone experiment). The low concentration dispersant alone

treatment was prepared in a single batch of sterile GP2 (14 L) spiked with approximately 20 µL of C9500. The batch was mixed for 30 minutes and, subsequently, 100 mL aliquots were transferred into the shake flasks.

Oil Components and Dispersant Analysis

To monitor C9500 degradation, DOSS was measured using liquid chromatography tandem mass spectrometry (LC-MS/MS) following the Standard method ASTM D7730−11 (2011). For the high concentration experiment, oil analysis was conducted with a 6890 GC coupled with a 5973 mass spectrometry from Agilent (Palo Alto, CA). In the low concentration experiment, an Agilent (Palo Alto, CA) 7000 GC Triple Quad system was used. The quantification range for the single quad and triple quad mass spectrometry are 1-30 µg/L and 0.5-10 µg/L, respectively. The same column, a DB-5MS column (30m × 0.25mm, 0.25µm film thickness), was used to achieve chromatographic separation of analytes in both instruments. The method was a modification based on EPA Method 8270D (2007). Details of sample preparation methods and oil extraction by DCM can be found elsewhere (Campo et al., 2013). The targeted oil components were pristane (PR), phytane (PH), normal paraffins (n-C_{10-35}) and 2-, 3- and 4-ring PAH compounds and their alkylated homologues (C_{0-4}-naphthalenes, C_{0-3}-fluorenes , C_{0-3}-dibenzothiophenes, C_{0-4}-phenanthrenes/anthracenes, C_{0-4}-napthbenzthiophenes, C_{0-2}-pyrenes, C_{0-4}-chrysenes). The concentrations of these analytes were normalized to the concentration of hopane present in the oil (Prince et al., 1994).

Statistical Analysis

Statistical analysis was performed with Graphpad Prism 6 software. Lag phases were determined by two-way ANOVA followed by Tukey's test among different sampling events. First-order rate

coefficients were calculated by least squares. To detect significant difference between two treatments, extra sum-of-square F test (for first order mechanism) and an equivalent form (Jerrold, 1984) of analysis of covariance (for zero order mechanism) were conducted. The Student *t*-test was used when comparing two groups of values (i.e. concentration values of two sampling events). In all the aforementioned tests, a significance level $\alpha = 0.05$ was used.

RESULTS AND DISCUSSION

Removal of DOSS

High Concentration Experiment. Biologically active samples revealed a rapid removal rate of DOSS at 25 °C (Fig. 1A-B, open symbols) with extents of transformation exceeding 95% after 2 d in dispersed oil (Fig. 1B) and 4 d in C9500 alone (Fig. 1A). The first order rate coefficients were -1.82 ± 0.25 d^{-1} (ANS+C9500) and -0.71 ± 0.024 d^{-1} (C9500 alone). The presence of oil enhanced the DOSS biodegradation rate constant by a factor of approximately 2.6. Disappearance of DOSS was also observed in KCs at 25 °C, with zero order rates of -138 ± 7 and -221 ± 11 $\mu g\ L^{-1}\ d^{-1}$ for C9500 alone (Fig. 1C) and dispersed oil (Fig. 1D), respectively. As monooctyl sulfosuccinate (MOSS) was found in KCs, Campo et al. (2013) explained this abiotic loss in terms of hydrolysis which was confirmed by (Batchu et al., 2014). The presence of oil significantly enhanced DOSS removal in KCs ($p < 0.0001$).

In contrast with the rapid removal rates observed for DOSS at 25 °C, the biotic and abiotic processes at 5 °C were much slower. DOSS persisted for 40 d before its concentration decreased in all treatments. The difference in DOSS concentration between live samples and parallel KCs in the dispersed oil treatment was statistically insignificant at days 48 ($p = 0.078$) and 56 ($p = 0.726$). In the C9500 alone treatment, DOSS was significantly lower in live samples

than in KCs at days 48 ($p = 0.0015$) and day 56 ($p = 0.023$). These results point to biotransformation as the predominant DOSS removal mechanism as opposed to hydrolysis.

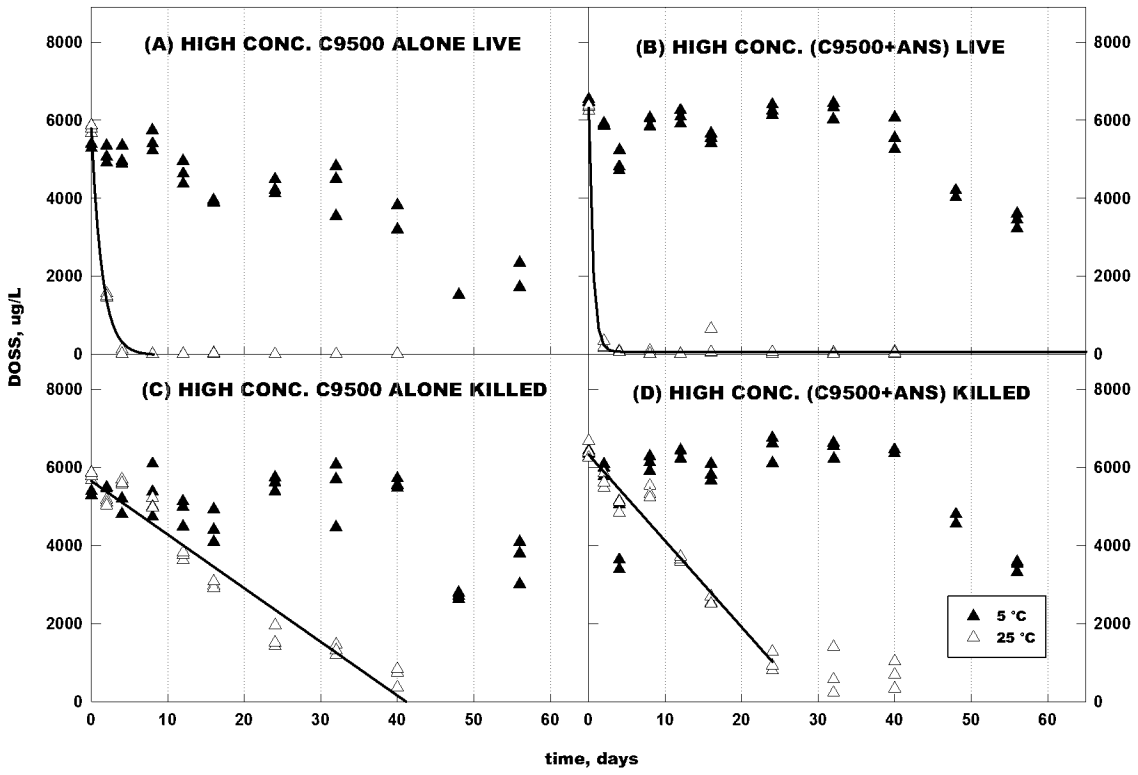

Figure 1. Disappearance of DOSS in high concentration experiment in the absence (A, C) and Presence (B, D) of ANS at 5 °C and 25 °C. Live samples are in panel A and B, whereas killed controls are in panels C and D.

Low Concentration Experiment. At 25 °C and for the dispersed oil treatment, no lag period was observed in DOSS uptake, which showed a first-order constant rate of -0.16 ± 0.015 d^{-1} (Fig. 2B). In the absence of oil (Fig. 2A), we observed an acclimation period of 2 d followed by DOSS biodegradation (-0.07 ± 0.009 d^{-1}). The presence of oil favored DOSS removal as was observed in the high concentration experiment. Removal extents over 95% for DOSS required 32 and 48 d in the presence and absence of oil, respectively. In the corresponding KCs, DOSS concentrations decreased following zero-order kinetics, with constant rates of -4.1 ± 0.3 μg L^{-1} d^{-1} (C9500 alone, Fig. 2C) and -5.1 ± 0.4 μg L^{-1} d^{-1} (C9500 and ANS, Fig. 2D).

For the high concentration experiment at 25 °C and regardless of the presence of oil, the

time series concentration of DOSS in the live samples rapidly declined to zero and clearly separated from the values measured in the corresponding KCs. In the low concentration experiment, it appeared that DOSS disappearance with time in both treatments, C9500 alone and dispersed ANS, overlapped with the abiotic losses observed in the KCs (Fig. 2). To differentiate between microbial activity and hydrolysis, we compared by a *t*-test ($\alpha = 0.05$) the average DOSS concentrations measured in live samples with those values found in the parallel KCs. Live samples significantly departed from the controls after 4 d ($p = 0.0227$) and 16 d ($p = 0.0053$) in the presence and absence of ANS, respectively. At early stages in the experiment, we could not identify the predominant removal mechanism for DOSS but, eventually, microbial uptake prevailed as the main process in terms of rate and extent.

DOSS biodegradation was at both the higher initial concentration and temperature ???????????. Nevertheless, based on our low concentration results, it is reasonable to infer that biodegradation of DOSS at the surface of GOM might not happen as rapidly since dilution and emulsification can attenuate the concentration of dispersant. In this fashion, DOSS was detected with a reporting limit of 0.25 $\mu g\ L^{-1}$ and a highest concentration of 229 $\pm$16 $\mu g\ L^{-1}$ in surface water samples collected 1-2 months after the spill near the Macondo well (Gray et al., 2014).

DOSS concentration remained unchanged (ANOVA followed by Tukey's test among all sampling events in each treatment) in live samples and KCs under 5 °C and low oiling conditions (Fig. 2, closed symbols). Such finding agreed with studies by Kujawinski et al. (2011) and Gray et al. (2014) who concluded that subsurface degradation of DOSS was minimal. Campo et al. (2013) observed no hydrolysis or biodegradation of DOSS in SLC at 5 °C within 42 d.

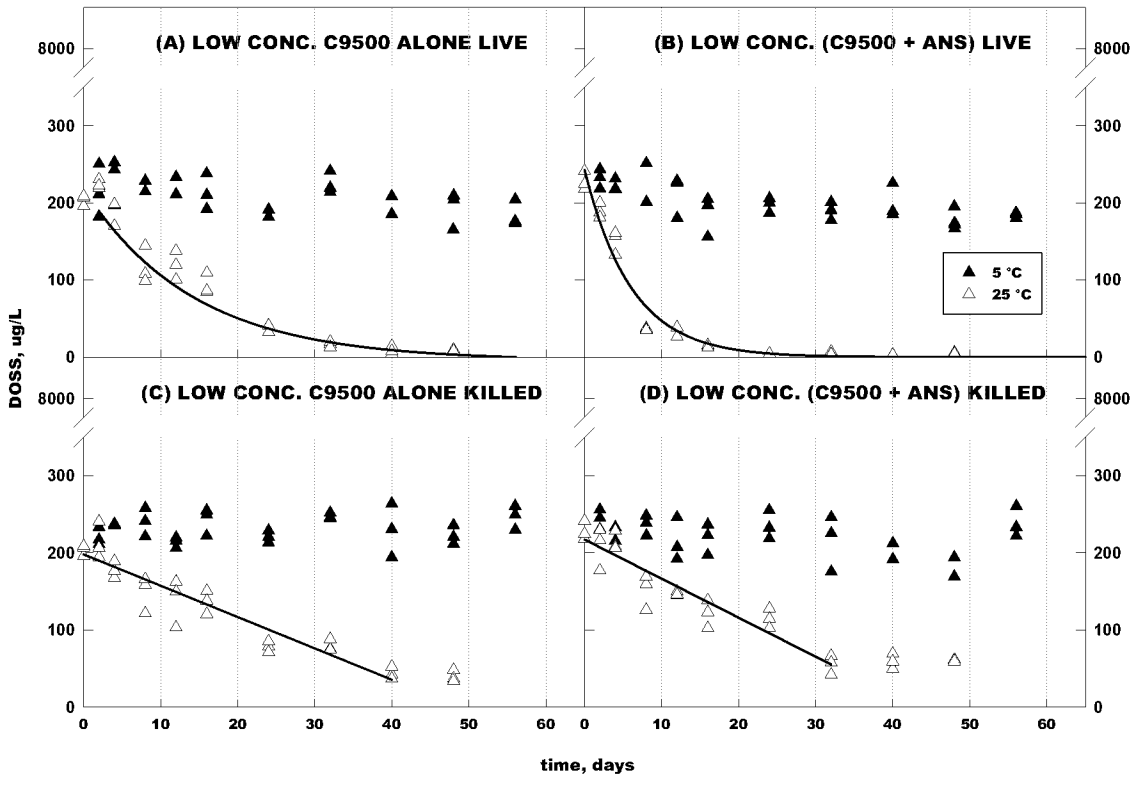

Figure 2. Disappearance of DOSS in low concentration experiment in the absence (A, C) and Presence (B, D) of ANS at 5 °C and 25 °C. Live samples are in panel A and B, whereas killed controls are in panels C and D.

We also included a series of dispersed oil KCs containing a protease cocktail (Fig. 3A), which inhibits enzymatic activity to clarify the abiotic DOSS disappearance. At 25 °C, the addition of inhibitor clearly slowed down the hydrolysis process as the zero-order rate constant in the protease KCs was -3.7 ± 0.4 μg L^{-1} d^{-1} (Fig. 3A) as opposed to -5.1 ± 0.4 μg L^{-1} d^{-1} (Fig. 3B) obtained for the regular KCs (p = 0.021). Such finding confirms the enzymatic nature of DOSS hydrolysis. Nevertheless, different enzymes may be involved in the process so that the protease cocktail could not block all of them. In the protease controls at 5 °C, DOSS persisted throughout the experiment as observed in the regular KCs for that temperature. This indicates that either the lower temperature attenuated DOSS breakdown, the cryo culture lacked the

required enzymes, or a combination of both causes???????????.

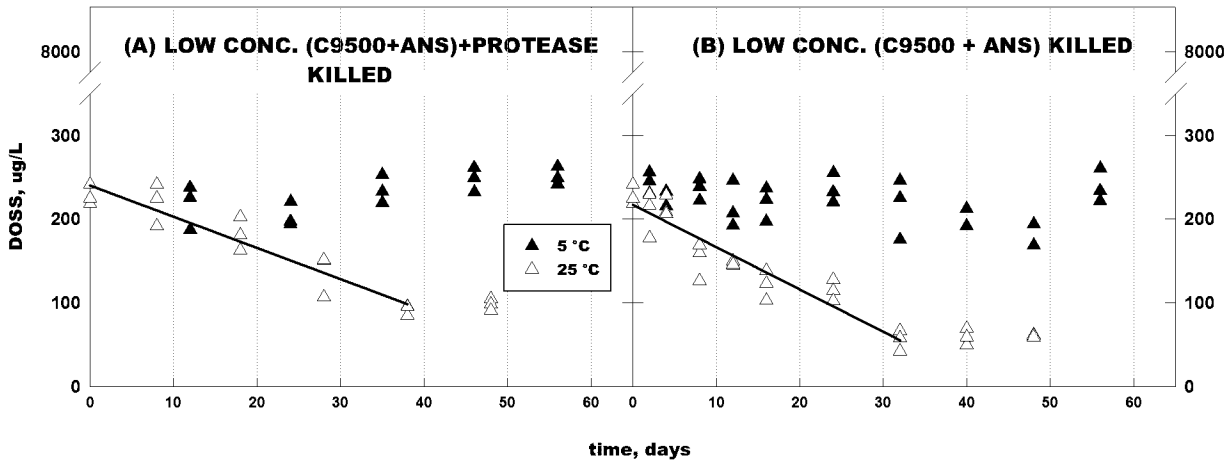

Figure 3. Disappearance of DOSS in low concentration dispersed oil killed controls in the presence (A) and absence (B) of protease at 5 and 25 °C.

Total Alkane Degradation

High Concentration Experiment. Total alkanes at both temperatures are presented in Fig. 4 A-B. At 25 °C, the meso culture metabolized the aliphatic fraction with a first-order rate constants of -0.91 ± 0.10 and -0.85 ± 0.04 d^{-1} in the absence and presence of C9500, respectively. For both treatments, the extent of removals reached 99% after 4 days. As expected, aliphatics degraded slower at 5 °C. In the dispersed oil treatment, this fraction followed first order kinetics (-0.24 ± 0.01 d^{-1}) while, for the oil alone samples, alkanes exhibited a linear decay during the first 8 d followed by first order kinetics of similar rate (-0.25 ± 0.03 d^{-1}). After days 16 (dispersed oil) and 24 (oil alone), the alkane extent of removal was 99% for the lower temperature.

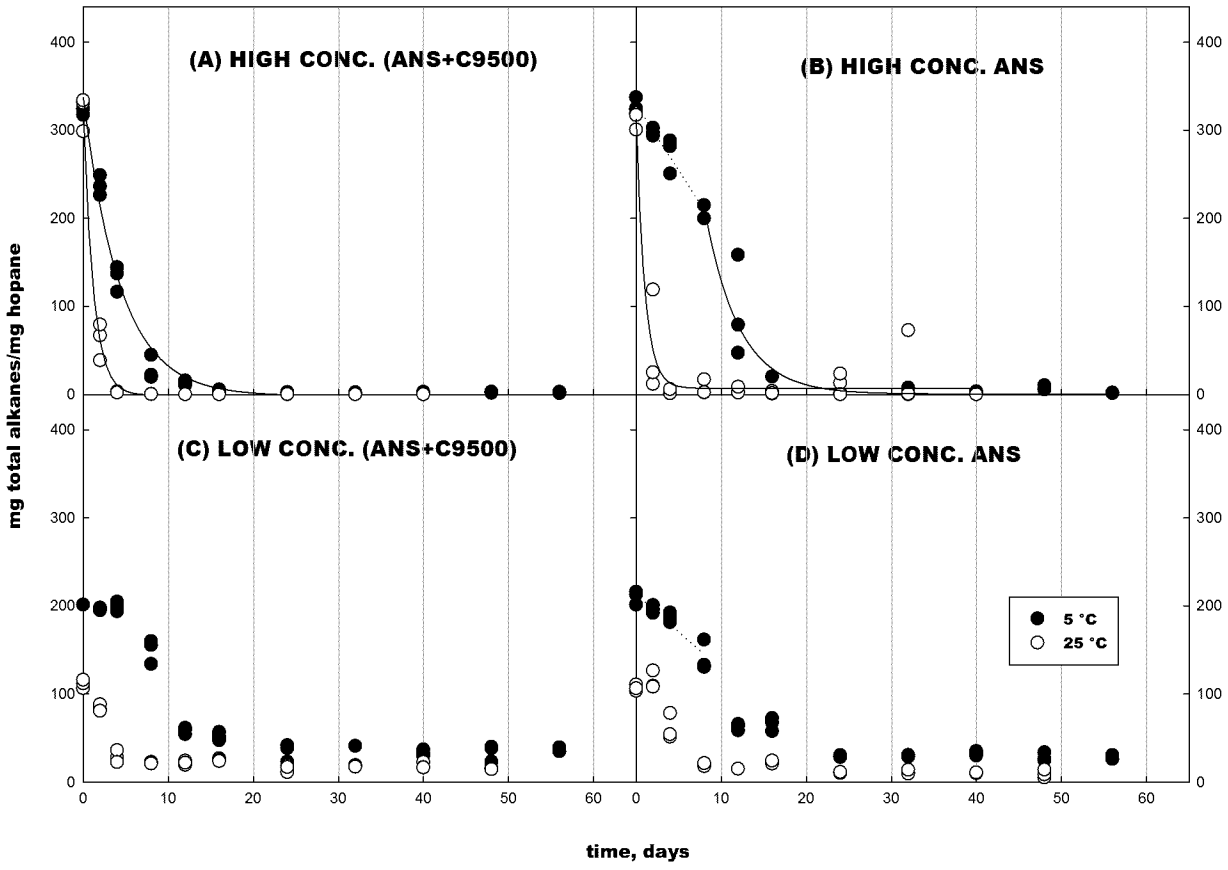

Figure 4. Biodegradation of hopane-normalized total alkanes at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D).

Low Concentration Experiment. In this experiment, the initial total alkane concentrations were considerably different at 5 and 25 °C (Fig. 4 C and D). The meso culture showed background concentrations of PAHs and hopane, the conservative biomarker to which all the target analytes concentrations are normalized. The hopane background levels were comparable to those measured in the initial oil added to the flasks. Hence, at time zero, the total alkane concentration normalized to hopane at 25 °C were almost half of the corresponding value at 5 °C.

At 25 °C, the alkane residual concentrations ranged from 8 to 18% of the initial values (Fig. 4 A and B). In the oil alone treatment, alkane biodegradation started after a lag phase of 2 d, while these chemicals were promptly metabolized in the dispersed oil treatment. At 5 °C, the cryo culture required 4 d for acclimation in the dispersed oil. In the absence of dispersant, after a slow and steady linear decay, fast disappearance of alkanes occurred between days 8 and 12. This suggests that C9500 reduced the length of the acclimation phase for alkane uptake.

Table 1 summarizes the actual concentrations of total alkanes (not normalized to hopane) for the first and last sampling event for both the high and low concentration experiments. It is noteworthy that a higher alkane residual amount was observed in the low concentration experiment than the high concentration one.

Table 1. Average and standard deviations (sd) of total alkanes and PAHs concentration in the high and low concentration experiment at first and last sampling event.

			5 °C		25 °C		5 °C		25 °C	
Samples			**ANS alone**		**ANS alone**		**ANS+C9500**		**ANS+C9500**	
			average (µg/L)	**sd**	**average (µg/L)**	**sd**	**average (µg/L)**	**sd**	**average (µg/L)**	**sd**
High Conc.										
	Alkanes	**First**	47,400	604	43,300	9,390	24,600	973	27,200	126
		Last	201	26	36	42	186	52	BDL	BDL
	PAHs	**First**	14,500	110	13,700	3,090	7,310	323	8,790	94
		Last	3,040	281	2,470	300	1,300	74	1,270	158
Low Conc.										
	Alkanes	**First**	2,010	202	1,740	149	1,420	72	1,130	39
		Last	216	21	83	75	225	13	142	4
	PAHs	**First**	482	53	773	25	330	12	453	14
		Last	71	5	88	5	58	10	80	4

Degradation of Individual Alkanes

High Concentration Experiment. Table S3 summarizes the first-order biodegradation

coefficients for individual alkanes. At 25 °C, among all the compounds monitored only n-C_{10} biodegraded faster (-2.00 vs. -1.34 d^{-1}) with the addition of dispersant. Branched alkanes exhibited similar rates regardless of the presence of C9500, although higher variability among triplicates was observed in the oil alone samples (Fig. S1 A and B). At 5 °C, consistently higher rates for n-$C_{10\text{-}27}$ were observed in the dispersed oil treatment when compared to the oil alone treatment. Also, no lag period for the biodegradation of branched alkanes occurred when C9500 was added, whereas an 8-day lag period occurred in the absence of dispersant.

Low Concentration Experiment. As depicted in Figs. S1-5 (panels C and D), only n-$C_{10\text{-}16}$?????was completely removed at both temperatures in the low concentration experiment. In this case,a more qualitative approach was used by comparing the disappearance time. At 25 °C in the low oil concentration experiment, n-C_{10} was degraded faster in the presence of dispersant, disappearing completely before day 2, whereas in the absence of C9500, its depletion was only achieved after day 8. The biodegradation of n-C_{10} was also accelerated when C9500 was present at 5 °C, as shown in Table S4 (-0.47 vs -0.63 d^{-1}). At both temperatures, branched alkanes were not degraded faster in the presence of dispersant, as shown in Table S4 and Fig. S1 C-D.

The major mechanism of uptake of water-insoluble substrates (i.e., alkanes) is generally recognized as direct interaction between microbes and the hydrophobic substance (Rojo, 2009; Wentzel et al., 2007) and the effects of surfactant on such process are inconclusive (Bredholt et al., 2002; Bruheim et al., 1999; Tang et al., 1998). Both conducive and inhibitory effects of dispersant on oil biodegradation have been reported (Lindstrom and Braddock, 2002; Prince et al., 2013; Venosa and Holder, 2007). In this study, C9500 exhibited positive effects on the biodegradation of alkanes in the high and low concentration experiments. Such effects included enhancement of biodegradation rates and shortening of lag periods. Rate enhancements were

more notable in the high concentration experiment, whereas C9500 clearly shortened lag phases in the lower concentration tests.

The application of dispersants to an oil slick promotes the formation of small oil droplets by decreasing the interfacial tension between oil and water (Jasper et al., 1978; Li et al., 2009; Lunel, 1995). Consequently, the total interfacial area of oil droplets for microbes to interact with is increased. In the high concentration experiment, exposure of substrates to the microbes is enough that biodegradation can initiate soon after inoculation. In this case, it is likely that dispersants assisted degradation mainly by increasing interfacial area for microbes to interact with oil. For the low concentration experiment, the observed long acclimation phase at both temperatures was possibly due to limited exposure of alkanes to the microorganisms in the beginning. Since the oil load, appearing as an iridescent sheen, is so low, the mixing energy in the flasks could be high enough to produce similar droplet size distribution in the oil alone and dispersed oil treatments.

Total Aromatics Degradation

High Concentration Experiment. Fig. 5A-B presents total PAH biodegradation data for the high concentration experiment at both temperatures. Aromatics removals of 42 and 24% occurred by day 2 in the dispersed oil and the oil alone treatment, respectively. This difference in extent was significant ($p < 0.0001$), which indicates a positive effect of the dispersant. At 5 °C, the time for attaining maximum level of PAHs removal in non-dispersed treatment took twice as long as in dispersed oil treatment 16 days vs. 8 days (solid symbols in Fig. 5B and 5A, respectively). The overall PAHs removals in the presence and absence of dispersant were 86% and 82%, respectively.

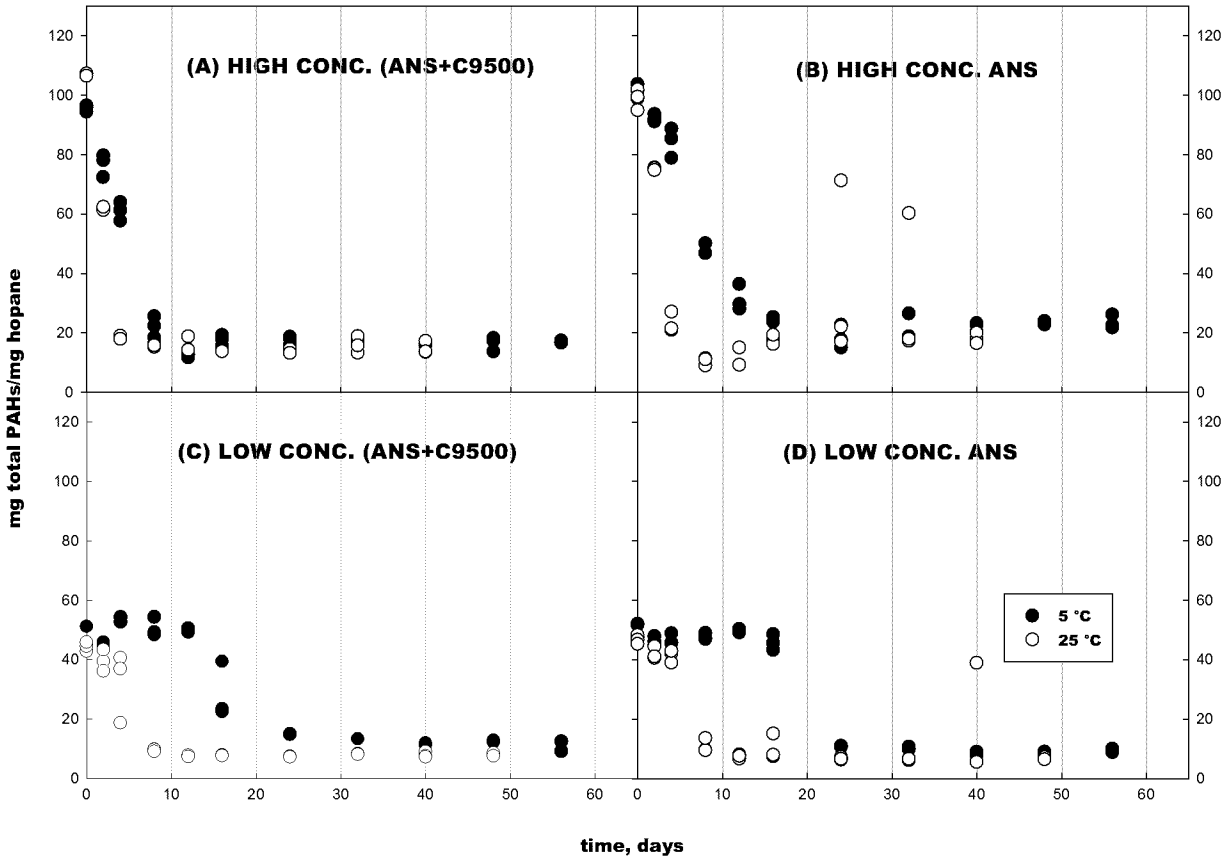

Figure 5. Biodegradation of hopane-normalized total PAHs at both temperatures in high concentration treatments (A, B) and low concentration treatments (C, D).

Low Concentration Experiment. As shown in Fig. 5C-D, when comparing the two low concentration treatments at 25 °C (open symbols), the only difference was in one replicate of dispersed oil on day 4, which had significantly lower total PAH concentration than the parallel diluted oil alone samples. At the high temperature (open symbols), the patterns of PAH degradation were similar in the absence and presence of C9500, regardless of initial concentration. The treatment without dispersant exhibited higher variability. The lag period at the low temperature (closed symbols) was longer than at 25 °C; it lasted 12 and 16 days for dispersed oil and oil alone, respectively. For the sampling event on day 16, the extent of PAH removal was 20 to 50% in the replicates of dispersed oil sample, while it was negligible in the

treatment without dispersant.

Transport of compounds from non-aqueous phase to aqueous phase is often the most rate-limiting step in the biodegradation of PAHs. Surfactants can enhance the dissolution of PAHs by increasing interfacial area of oil droplet and subsequently increase the bioavailability of PAHs to microorganisms (Haritash and Kaushik, 2009; Mrozik et al., 2003). The effect of C9500 on the biodegradation of total PAHs was less pronounced in the low concentration experiment than in the high concentration experiment, as this effect was observed only in one or more replicates in a single sampling event. This finding is consistent with our previous assumption about low concentration experiment: droplet size distribution in the oil alone and dispersed oil treatments are similar. Dispersion in the oil alone microcosms might have been efficient enough even without the addition surfactant, considering the mixing energy was sufficient (Kaku, 2006) and the initial presence of oil was an iridescent rainbow-colored sheen. Clearly, the comparison between our low concentration dispersed oil samples and oil alone samples indicates that dispersants had little effect on the concentration of PAHs. Nevertheless, most toxicity studies on dispersed oil conclude that the presence of dispersant increased environmental risk for aquatic organisms living in the water column (Milinkovitch et al., 2011; Ramachandran et al., 2004), which is possibly due to their relatively high concentration of oil (4.75 g/L and 100 mL/L, respectively) and dispersant (0.25g/L and 5 mL/L, respectively) in the laboratory tests.

Individual Aromatics Degradation

High Concentration Experiment. A first-order model could be used to fit the biodegradation of the individual PAHs that had a negligible fraction persisting by the end of the high concentration experiment. At both temperatures, the biodegradation rates of naphthalene,

phenanthrene, fluorine, and dibenzothiophene and their homologues in the high concentration experiment were consistently higher in the presence of dispersant (Table S5). The greatest enhancement was observed with naphthalene at 25 °C, which was 2.9-fold higher than at 5 °C (-1.94 d^{-1} vs. -0.68 d^{-1}). The surfactant likely assisted the transfer of more soluble compounds from the oil phase to the aqueous phase through the formation of small oil droplets and the resultant increase in interfacial area. Because the bio-uptake of aromatics is mostly from aqueous phase (Haritash and Kaushik, 2009; Mrozik et al., 2003), C9500 promoted the biodegradation of PAHs by increasing their bioavailability in water.

Low Concentration Experiment. Among the low concentration individual PAHs, the time-varying concentration of specific aromatics could be fitted to a first-order kinetics model (rates shown in Table S6), and the results revealed reduced rates in low concentration treatments, especially when C9500 was present (i.e. naphthalene: -0.36 d^{-1} v -1.94 d^{-1}). However, we could not fit a first-order model for the other PAH compounds in the low concentration experiments because of the long lag period and the immediate and nearly complete removal after biodegradation started. As mentioned before, the impact of C9500 in the low concentration experiment was difficult to assess, because different PAH concentrations in parallel dispersed oil and oil alone samples was observed only at one or more replicates in a single sampling event (25 °C: day 4, 5 °C: day 16). In the low concentration experiment, the starting PAH concentrations (330-773 **µg/L**) were below the remaining residual found in the high concentration experiment (1270-3040 **µg/L**), but the cultures were able to degrade the aromatic compounds to an even lower level, as shown in Table 1. C_4-naphthalene, $C_{2\text{-}4}$-phenanthrene, $C_{2\text{-}3}$-fluorene, and naphthobenzothiophene were removed at higher extent in the low rather than in the high concentration samples (Fig. S6). The increase in removal extent for the aforementioned

aromatics were 6-14%, 4-47%, 14-54%, and 55-86% respectively. Zahed et al. (2010) also observed similar enhancement of crude oil removal extent in low oiling experiments. Taking C_2-phenanthrene as an example, it was fully degraded in the low concentration experiment, whereas its removal extent ranged from 66% to 94% when the initial oil concentration was high. The enhancement in the extent of removal under diluted conditions was more remarkable at low temperature, which contributed to our conclusion that solubility was the limiting factor for those compounds to be biodegraded. Whether oil concentration is high or low, pyrene, chrysene and naphthobenzothiophene homologues persisted until the last sampling event at both temperatures, as depicted in Figs. S7 and S8. The heavy PAHs, such as chrysene, are likely to be retained on a beach for several years, according to Yin et al. (2015), who conducted research that monitors the submerged oil mats and surface residual oil balls off Alabama's beach affected by the 2010 DWH incident.

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Microbial response to the MC-252 oil and Corexit 9500 in the Gulf of Mexico

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The Deepwater Horizon spill released over 4.1 million barrels of crude oil into the Gulf of Mexico. In an effort to mitigate large oil slicks, the dispersant Corexit 9500 was sprayed onto surface slicks and injected directly at the wellhead at water depth of 1,500 m. Several research groups were involved in investigating the fate of the MC-252 oil using newly advanced molecular tools to elucidate microbial interactions with oil, gases, and dispersant. Microbial community analysis by different research groups revealed that hydrocarbon degrading bacteria belonging to *Oceanospirillales*, *Colwellia*, *Cycloclasticus*, *Rhodobacterales*, *Pseudoalteromonas*, and methylotrophs were found enriched in the contaminated water column. Presented here is a comprehensive overview of the ecogenomics of microbial degradation of MC-252 oil and gases in the water column and shorelines. We also present some insight into the fate of the dispersant Corexit 9500 that was added to aid in oil dispersion process. Our results show the dispersant was not toxic to the indigenous microbes at concentrations added, and different bacterial species isolated in the aftermath of the spill were able to degrade the various components of Corexit 9500 that included hydrocarbons, glycols, and dioctyl sulfosuccinate.

Keywords: MC-252, oil, biodegradation, Corexit 9500, hydrocarbon, dispersant, Gulf of Mexico

DEEPWATER HORIZON OIL SPILL

In April 2010, high-pressure oil and gas caused the Deepwater Horizon drilling rig in the Gulf of Mexico to explode making it the worst oil spill in the United States and the largest marine oil spill in the history of the petroleum industry. MC-252 oil was released from the broken riser pipe at a depth of 1,500m below surface. Approximately 4.1 million barrels of light crude oil was released into the Gulf waters, of which a significant amount has been accounted for in the cleanup effort (including siphoning, controlled burns, skimming, booming; Atlas and Hazen, 2011). The remaining fraction along with the added dispersants, contributed to the sudden influx of aliphatic and aromatic hydrocarbons in the Gulf water leaving a plume (cloud of dispersed oil droplets), more than 35km in length (Camilli et al.,2010),that significantly impacted indigenous microbial population. Some fraction of the oil eventually made its way to the beaches, marshes, and sediments. Results from several research groups indicated that the oil degrading indigenous microbes played a significant role in reducing the overall environmental impact of the oil spill (Hazen et al., 2010; Valentine et al., 2010; Atlas and Hazen, 2011; Redmond and Valentine, 2011; Mason et al., 2012).

MICROBIAL RESPONSE TO MC-252 OIL IN THE DEEP OCEAN WATERS

As the mixture of aromatic (monocyclic and polycyclic) and aliphatic hydrocarbons in MC-252 oil moved through the water column, it was subjected to chemical and physical partitioning. A recent study combining atmospheric, surface, and subsurface chemical data has stated that the readily soluble hydrocarbon components constituted approximately 70% of the deep plume mass and that the remaining traveled as trapped oil droplets throughout the water column (Ryerson et al., 2012). As the spill events progressed, the microbial community changed in response to the available hydrocarbons. Shortly after the spill in May 2010, bacterial counts in the plume were significantly higher (5.5 ffi 10^4 cells/mL) than outside the plume (approximately 2.7 ffi 10^4 cells/mL; Hazen et al.,2010). The rapid response of different groups of bacteria may imply differential utilization of nutrients/hydrocarbons introduced by the spill (Atlas and Hazen, 2011; Valentine et al., 2012). In the initial stages in May and June 2010, microbial community composition in the plume waters was highly enriched in *Gammaproteobacteria* (Hazen et al., 2010; Redmond and Valentine, 2011). 16S ribosomal RNA based PhyloChip microarray and 16S rRNA gene based clone libraries identified *Oceanospirillales* as dominant microbes (Hazen et al.,2010) but also found 15 other *Gammaproteobacteria* taxa that were enriched by the subsurface plume. Functional gene based GeoChip microarray analysis revealed significant increases in expression of more than 1600 genes involved in hydrocarbon degradation (BTEX, alkane, cycloalkanes, and PAH) over background non-plume samples. In addition, genes for carbon metabolism, nitrogen assimilation, sulfate reduction, phosphorus release, metal resistance, and bacteriophage replication were higher in abundance in plume waters, along with several functional genes derived from *Oceanospirillales*. In absence of an isolate, deep sequencing of community DNA and RNA and single-cell genomics provided greater insights into *Oceanospirillales* for genes and pathway supregulated by the spill (Mason et al.,2012). Genes involved in alkane degradation (specifically, cyclohexane) were expressed

along with genes for nutrient uptake, motility, and chemotaxis. Together, they might have enabled cells of this microbial group to colonize, feed on the oil, and multiply in numbers in the plume (Mason et al., 2012).

Clone library analysis of 16S rRNA gene showed dominance of several sequences mostly related to *Cycloclasticus* and *Colwellia* in samples collected from the deep plume in June 2010 (Valentine et al., 2010; Redmond and Valentine, 2011). Stable isotope probing experiments with 13C-labeled gaseous substrates showed that *Colwellia* were likely consuming propane, ethane, and potentially butane. *Cycloclasticus* were thought to be consuming BTEX compounds that were the primary oil constituents found in the subsurface plume during this time period (Redmond and Valentine, 2011). 16S rRNA gene cloning and sequencing of plume samples collected in September 2010 by Kessler et al. (2011) reported a very different microbial community structure, containing high numbers of methylotrophic bacteria (*Methylococcaceae*, *Methylophaga*, and *Methylophilaceae*) and low abundance of *Colwellia*, *Cycloclasticus*, and *Oceanospirillales* – which had dominated previously. The authors attributed this change to a residual bloom of methanotrophic activity having occurred in July 2010. 16S rRNA clone libraries identified methanotrophs, methylotrophs, *Flavobacteria*, and *Alphaproteobacteria* (*Rhodobacterales*) to be relatively abundant in plume waters sampled later in September 2010 (Redmond and Valentine, 2011).

In contrast to bacterial community composition, archaeal community in plume samples did not show much deviation from May through September 2010 (Redmond and Valentine, 2011). Marine Group II *Euryarchaeota*, other marine *Euryarchaeota*, and *Thaumarchaeota* were consistently present, and it is unlikely these archaeal groups had any role in degradation of the oil hydrocarbons (Redmond and Valentine, 2011). Moreover, ammonia oxidation and nitrification by *Nitrosopumilus maritimus* belonging to marine Group I *Thaumarchaeota* was only slightly impaired when amended with 10 or 100 ppb oil (Urakawa et al., 2012). These results suggest that the sudden outpouring of oil hydrocarbons from the spill had no significant effect on the marine archaeal population.

Seventeen *Vibrio* isolates representing five distinct genotypes were isolated in April and May 2010 from ocean water, sediment, and oysters in coastal Louisiana (Smith et al., 2012). While some of these *Vibrio* isolates grew on surfactants Tween 40 and Tween 80, none of them were able to use PAH such as naphthalene and phenanthrene (Smith et al., 2012). *Vibrio* strain S4BW isolated from the surface waters 6 weeks after the spill (Gauglitz et al., 2012) was able to produce siderophores to better sequester limiting nutrients like Iron. Several *Vibrio* species belonging to *Gammaproteobacteria* were also isolated from plume water and contaminated Elmers beach samples (Chakraborty, R., unpublished). DNA based dot blot hybridization using a specific probe detected greater than 10^5 CFU/g of *Vibrio vulnificus* in tar balls and sands from beaches in Mississippi and Alabama collected from July through October 2010 (Tao et al., 2011). *Vibrios* are common inhabitants of ocean water (Grimes et al., 2009) and have been associated with hydrocarbon degradation (Hedlund and Staley, 2001; Thompson et al., 2004). Although they are ubiquitous in marine environments, it is unlikely that they were major players in the biodegradation of the oil as they were not amongst the most enriched microbes in the plume or in the coastal contaminated area.

MICROBIAL RESPONSE TO MC-252 OIL IN LABORATORY INCUBATIONS

By stable isotope probing experiments with 13C-labeled methane, ethane, propane, or benzene in laboratory incubations followed by 16S rRNA gene clone libraries, Redmond and Valentine (2011) demonstrated that *Colwellia* increased in abundance during enrichment on these gases at cold temperatures (4°C). This implied that temperature was a major determinant in selection of this group of microorganisms in the plume. Further, laboratory-based incubations with MC-252 oil, Corexit, and water outside the plume also revealed an increase in abundance of *Colwelliaceae* and *Oceanospirillales* (Bælum et al., 2012). *Colwellia* strain RC25 was isolated from these laboratory incubations that rapidly degraded 75% of the initial oil added in 10 days. 16S rRNA gene sequence of this strain showed 96% sequence similarity to the type strain, *Colwellia psychrerythraea* 34H and almost 99% similarity to the most abundant *Colwellia* species observed by 16S pyrosequencing in the original incubations. Interestingly, in these incubations, large flocs seemed to form with oil and/or Corexit, and detailed investigation indicated that *Colwelliaceae* were the dominant bacteria in the flocs. Flocs were absent from incubations amended with Iron. Apart from biomass, flocs contained oil and carbohydrates as revealed by Synchrotron radiation-based Fourier transform infrared (SR-FTIR) spectromicroscopy (Bælum et al., 2012). Flocs were also similarly observed from subsurface plume samples (Hazen et al., 2010) and on deepwater coral colonies near the Macondo well in November and December 2010 (White et al., 2012). It is likely that flocs contained oil, biomass, products of oil degradation, and other carbohydrates such as exopolysaccharides (Bælum et al., 2012; White et al., 2012).

MICROBIAL RESPONSE TO MC-252 OIL IN SURFACE WATERS

Surface water collected in June 2010 about 2–7 km away from the wellhead showed high microbial respiration, high hydrocarbon degradation and high rates of lipase, and alkaline phosphatase activity (Bethanie et al., 2011). Alkaline phosphatases are usually produced by microbes when challenged with phosphate starvation. Nitrate and Phosphate were added to water incubations to alleviate nutrient stress, and this seemed to increase microbial respiration and biomass (Bethanie et al., 2011). Clone library analysis of surface water collected in May and June 2010, 2–44 km from the Macondo wellhead demonstrated that microbial community composition differed dramatically from the deep-water plume sampled in the same time frame (Redmond and Valentine, 2011). *Cyanobacteria* and *Alphaproteobacteria* (SAR11 clade, *Rhodobacterales*, and *Rhodospirillales*) inhabited the surface waters with visible oil sheen, and *Gammaproteobacteria* (*Pseudoalteromonas*, *Pseudomonas*, *Vibrio*, *Acinetobacter*, and *Alteromonas* genera) were prevalent in samples that had heavy oil layer on top. Several *Cyanobacteria*, *Rhodobacterales*, and *Rhodospirillales* have been associated with oil hydrocarbon degradation (Brakstad and Lødeng, 2005; Hernandez-Raquet et al., 2006; Ibraheem, 2010), and several members of these bacterial groups are also capable of photosynthesis. Thus it is no surprise that they were abundant in the surface water. *Oceanospirillales*, *Colwellia*, and *Cycloclasticus*,

which were the most enriched microbial group in the deepwater plume samples (Hazen et al., 2010; Valentine et al., 2010; Redmond and Valentine, 2011; Mason et al., 2012) were an insignificant part of the total microbial community in these surface waters.

MICROBIAL RESPONSE TO MC-252 OIL IN SHORELINES AND MARSHES

A considerable amount of oil resulting from the spill impacted coastal waters of the Gulf of Mexico and washed ashore the marshes and the beaches (Allan et al., 2012). To better understand biodegradation of weathered and fresh oil in these environments, indigenous prokaryotic and eukaryotic microbial communities were studied. 18S rRNA gene based phylogenetic analysis was used to characterize eukaryotes inhabiting beach sediments prior to and following shoreline oiling (Bik et al., 2012). In this study, a substantial shift in communities between pre-spill and post-spill was reported. While sediments on the outer shores of Dauphin Island were greatly dominated by *Cladosporium* species (which can utilize hydrocarbon compounds extensively), marine *Alternaria* species dominated in brackish Mobile Bay waters. In addition, OTUs related to *Aspergillus*, *Acremonium*, *Acarospora*, *Rhodocollybia*, and *Rhizopus* species were reported in higher abundance in the post oil spill samples. A number of these marine fungal groups have been shown previously to metabolize hydrocarbon compounds (Bik et al., 2012) similar to those present in MC-252 oil as well.

In a study by Hore let al. (2012), mesocosms were initiated with MC-252 oil and sand from Dauphin Island to mimic the effect of oil spill on sandy beaches. The results confirmed that indigenous hydrocarbon degrading bacteria were present in beach sand and that the rate of oil degradation by these microbes were stimulated when amended with inorganic nutrients.

Culture-dependent and genomics-based studies were used to characterize microbial community in oil-contaminated and pristine sand samples collected from Pensacola beach in September 2010. Oil tainted sand harbored a higher microbial count of hydrocarbon degraders compared to pristine samples as corroborated by both Most Probable Number (MPN) and molecular methods. Based on initial DNA fingerprinting analysis, detailed Pyrotag sequencing of SSU rRNA amplicons revealed a significant community shift toward *Gammaproteobacteria*, and to a lesser extent the *Alphaproteobacteria*, following exposure to oil (Kostka et al., 2011). Furthermore, members of *Alcanivorax*, *Marinobacter*, *Vibrio*, *Pseudomonas*, *Pseudoalteromonas*, and *Acinetobacter* genera were isolated from the contaminated sample, several of which are known hydrocarbon degraders. In MC-252 oil-contaminated samples from Elmer's Beach, several such hydrocarbon degrading bacteria were isolated with representatives from the *Alcanivorax*, *Marinobacter*, *Pseudomonas*, *Roseobacter*, *Rheinheimera*, and *Vibrio* genera (Chakraborty R, unpublished). Metagenomic and metatranscriptomic analysis revealed an enrichment of a group of organisms within the *Rhodobacterales* family corresponding to samples with high total petroleum hydrocarbons (TPH; Lamendella, R., unpublished).

Sediment from coastal salt marsh in Alabama collected in June, July, and September 2010 were analyzed for structure and function of the native microbial community using PhyloChip and GeoChip microarrays (Beazley et al., 2012). Higher oil concentrations in samples from June and July corresponded to an increase in *Actinobacteria*, *Bacteroidetes*, and *Proteobacteria*. *Firmicutes* like *Bacilli* and *Clostridia* were more abundant in September when oil concentrations were lower. Oil concentration also influenced the community function, as the relative abundance of hydrocarbon degrading genes increased significantly when TPH concentrations were high, and decreased when hydrocarbons were low (Beazley et al., 2012).

Together, these data provide evidence that there exists an active aerobic microbial community indigenous to the shoreline environments that is capable of degrading petroleum hydrocarbons. In the event of an influx of these substrates, *in situ* microbial degradation is stimulated and mineralization of hydrocarbons is observed leading to natural attenuation. Rapid microbial respiration of readily accessible substrates also leads to oxygen-depleted subsurface environments. A study by Boopathy et al. (2012) with marsh sediments from Barataria Bay in Louisiana showed that microbial degradation of MC-252 oil occurred under anaerobic conditions as well, although the microbial community involved in the process was not discussed. The presence of *Rhodocyclaceae*, *Geobacteraceae*, and *Desulfobacteraceae* (as shown by 16S rRNA based PhyloChip) along with the detection of genes involved in anaerobic metabolisms such as sulfate reduction, nitrate reduction, and methanogenesis (as shown by functional gene microarray, GeoChip), also hinted toward occurrence of anaerobic hydrocarbon degradation in salt marsh sediments in coastal Alabama (Beazley et al., 2012).

MICROBIAL RESPONSE TO THE DISPERSANT COREXIT 9500

To prevent oil slicks, 1.8 million gallons of the dispersant Corexit 9500 and later Corexit 9527 was used both on surface and at the leaking wellhead. Most commercial dispersants typically contain one or more surfactant(s), with both hydrophilic and hydrophobic groups that encourage development of small oil-surfactant micelles (Hemmer et al., 2011). The resulting greater surface area enhances their entrainment in the water column and enhances bioavailability for microbial degradation while distributing the oil to lower concentrations aided by recirculation of water. However, while the concentrations and dispersant-to-oil ratios used in the MC-252 oil spill were reported to be much lower than the concentrations used for toxicity screenings (Kujawinski et al., 2011), not much is known about the persistence, toxicological effects, and the cumulative impact of dispersant with oil in Gulf of Mexico. In previous studies with microbial consortia at 8°C, addition of Corexit 9500 to fresh or weathered oil had not shown any change in oil degradation (Lindstrom and Braddock, 2002). However, microbial heterotrophs were present in significantly higher numbers in the presence of the dispersant, suggesting that Corexit 9500 provided an additional carbon source. Furthermore, phenanthrene was mineralized better when Corexit 9500 was added (Lindstrom and Braddock, 2002).

In the aftermath of the Deepwater Horizon oil spill, the effect of Corexit 9500 on bacterial viability was studied using isolates obtained from oil-contaminated sands from Elmers Island by Hamdan and Fulmer (2011). In general, Corexit 9500 decreased cell viability at all concentrations tested. At extremely low concentration (dilution of 1:1000), the dispersant seemed to promote cell viability of an isolate 99% similar to *Vibrio natriegens*

strain UST040801-014. At Corexit dilutions of 1:10, 1:25, and 1:50 (diluted with hexadecane), two hydrocarbon degrading isolates *Acinetobacter venetianus* and *Marinobacter hydrocarbon oclasticus* were severely affected, however the isolate most similar to *Pseudomonas pseudoalcaligenes* showed almost as much growth as the control at 1:50 dilution. Perhaps the presence of hexadecane helped alleviate the toxic effect of the dispersant in this case (Hamdan and Fulmer, 2011).

Corexit 9500 was composed of a mixture of hydrocarbons (50%), glycols (40%), and dioctylsulfosuccinate (DOSS; 10%). Not only did Corexit 9500 have no effect on the growth of microbial consortia enriched from the Gulf of Mexico, most of its components were biodegraded over time – the hydrocarbon fraction much more rapidly than the DOSS and glycol compounds (Bælum et al., 2012). *Colwelliaceae*, *Rhodobacteraceae*, *Oceanospirillales*, and *Actinobacteria* dominated the microbial community in these incubations containing 100ppm MC-252 oil and 60ppm Corexit 9500 from which *Colwellia* strain RC25 was isolated. Apart from this study with laboratory microcosm from our group (Bælum et al., 2012), there have been no other reports on the effect of Corexit 9500 on microbes such as *Colwellia*, that played a critical role in responding to the Deepwater Horizon oil spill.

To better understand the effect of the dispersant on this organism, experiments were initiated with active cultures of *Colwellia* strain RC25 in minimal media in the presence of MC-252 oil (100ppm) or MC-252 oil (100ppm) C Corexit 9500 (10ppm) at 4°C. Sacrificial samples were analyzed for TPH as described previously (Hazen et al., 2010; Bælum et al., 2012). Results indicate that oil was degraded faster in the treatments with dispersant

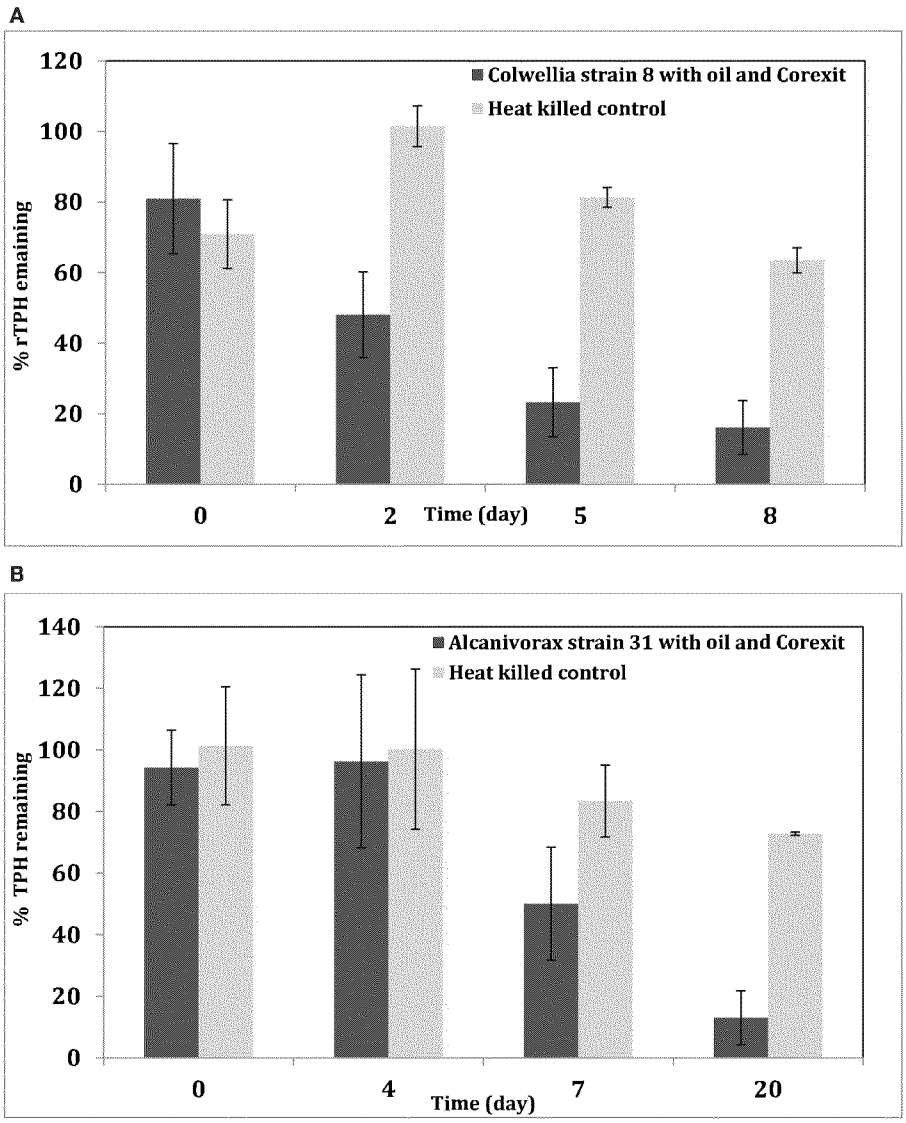

FIGURE 1 | (A) Degradation of total petroleum hydrocarbon (TPH) by *Colwellia* strain RC25 with 100 ppm MC-252 oil and 10 ppm Corexit 9500. **(B)** Degradation of TPH by *Alcanivorax* strain 31 with 20 ppm MC-252 oil and 1 ppm Corexit 9500.

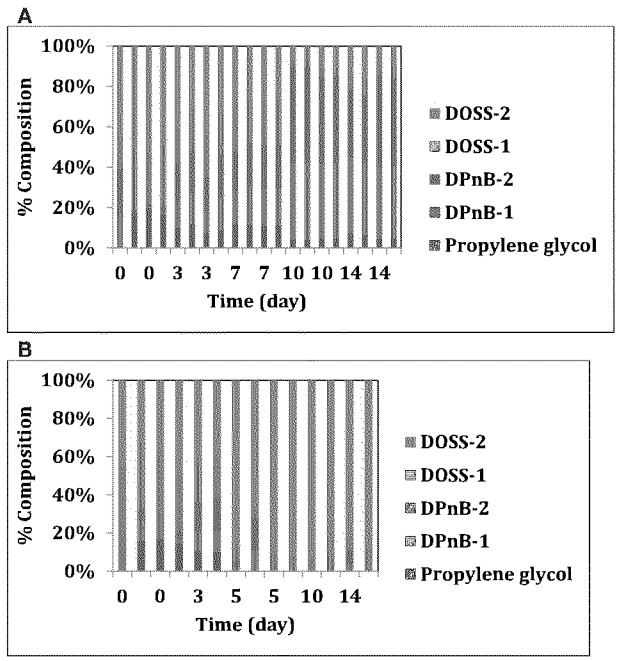

FIGURE 2 | (A) Degradation of Corexit 9500 compounds by *Colwellia* strain RC25. **(B)** Degradation of Corexit 9500 compounds by *Alcanivorax* strain 31.

(**Figure 1A**). This could be due to the fact that Corexit increases the solubility of oil in water and thus oil is more bioavailable for biodegradation. Similar results were obtained with an *Alcanivorax* strain isolated from oil-contaminated Elmers beach (**Figure 1B**) when tested with MC-252 oil (20ppm) with or without Corexit 9500 (1ppm). These two isolates belong to *Colwelliaceae* and *Oceanospirillales* respectively, which were the dominant microbial groups observed in the oil plume shortly following the spill, and our results confirm their role in biodegradation of the oil promoted by the addition of dispersant. In both experiments, oil (and Corexit) was the sole source of carbon.

We also looked deeper into the biodegradability of the dispersant itself by these two isolates. Corexit 9500 components were analyzed over time in sacrificial samples using methods described previously (Bælum et al., 2012). Five main compounds were quantified from Corexit 9500: propyleneglycol, two isomers of dipropylene glycol n-butyl ether (DPnB), and two isomers of DOSS. Both microbial isolates were able to degrade some components of Corexit 9500 (**Figures 2A,B**). While the glycol compounds DPnB present in Corexit 9500 were not degraded by *Colwellia* strain RC25, some isomers of DOSS were degraded within 19 days (**Figure 2A**). In support of this, a slow degradation of DOSS compounds was observed at plume depth after the oil spill (Kujawinski et al., 2011). *Alcanivorax* strain 31 was unable to degrade the DOSS components, but could degrade DPnB and propylene glycol components (**Figure 2B**). Differential degradation of the various components of Corexit 9500 by these two microbes suggests that complete mineralization could have been possible by consortia of the indigenous microbes that were enriched in the plume.

CONCLUSION

A systems biology approach closely integrated with chemical and statistical analyses fueled by interest from the scientific community, regulating agencies, and general public led to an unprecedented near real-time understanding of the fate of MC-252 oil degradation in the Gulf of Mexico (Chakraborty et al., 2012). The rapid response by the scientific community was greatly successful in documenting a comprehensive sequence of events resulting from the Deepwater Horizon oil spill. It was evident that microbes indigenous to the Gulf of Mexico waters were highly capable of mineralizing oil, and groups of microbes capable of degrading certain components of the oil hydrocarbons bloomed in sequence when those hydrocarbons were made available as substrates to them. Natural attenuation was partly facilitated by the addition of dispersant that increased the bioavailability of oil. While the dispersant was detrimental to the survival and health of different macro-organisms, representative microbes enriched from the plume were able to degrade oil better in its presence, and could further degrade certain components of the dispersant as well. Application of traditional microbiological methods with modern genome-based technologies led to an extensive understanding of how the deep-sea and shoreline microbial community responded. This provided an excellent opportunity for the scientific community to be able to predict microbial involvement in major oil spills in future.

ACKNOWLEDGMENTS

The authors would like to thank Jiawen Huang, Troy Hodges, and Darcie Long for their assistance in experiments with Corexit 9500. We would like to thank Yvette Piceno for critical reading of the manuscript. The research on dispersant was supported by a subcontract from the University of California at Berkeley, Energy Biosciences Institute (EBI), to Lawrence Berkeley National Laboratory under its U.S. Department of Energy contract DE-AC02-05CH11231.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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This article was submitted to Frontiers inMicrobiotechnology,Ecotoxicologyand Bioremediation,aspecialtyofFrontiersin Microbiology.

To: Craig Watts[craig@hydrosphere.net]; Holder, Edith[holder.edith@epa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov]
Cc: Peter Meyer[pmeyer@hydrosphere.net]; Cris Griffin[cgriffin@hydrosphere.net]
From: Barron, Mace
Sent: Fri 9/16/2016 3:31:21 PM
Subject: RE: Final report for recent round of toxicity tests

Thank you for the revised report and spreadsheet.

All looks good now.

We look forward to getting the Finasol report and spreadsheet.

Sincerely,

Mace

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Friday, September 16, 2016 8:40 AM
To: Barron, Mace <Barron.Mace@epa.gov>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Mace,

We have made the changes and corrections and renamed the report 16119 REV 091616. Here is the link to the revised report.

https://www.dropbox.com/s/conr0126ep4mepf/16119%20REV%20091616.pdf?dl=0

We have not received any Finquel. Finasol is the one we are wrapping up early next week.

Craig

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: Thursday, September 15, 2016 4:20 PM
To: Craig Watts <craig@hydrosphere.net>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Hey guys:

Just very few minor revisions requested from my technical review:

Table 2: A. Punctulata

* Acute column: please either spell out not applicable in the cell or add a footnote defining "NA".

*chronic column: replace NA with a footnote or something specifying the organism age or life stage tested.

Table 12:

*report the NOEC and IC24 values in uL/L

Please do provide a revised copy, as well as a revised excel sheet with the toxicity summary tables.

Thanks again for your work with EPA and Pegasus.

PS: also, could you update us what is next on your schedule for this work (e.g., finquel? Anything else to be completed from testing samples we have provided?

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Thursday, September 15, 2016 2:35 PM
To: Barron, Mace <Barron.Mace@epa.gov>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Mace,

Spreadsheets? You have a beautiful report in front of you!

Here is your spreadsheet.

Craig

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: Thursday, September 15, 2016 2:56 PM
To: Craig Watts <craig@hydrosphere.net>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Thank you!

I was able to download a copy and will provide a technical review in next few days.

Could you also provide a copy of just the tox results in excel format similar to what you provided for the dilbits (attached).

Much appreciated,

Mace

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Thursday, September 15, 2016 1:49 PM
To: Barron, Mace <Barron.Mace@epa.gov>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: Final report for recent round of toxicity tests

To all,

So much for our effort to simply and streamline the reports. The Corexit report weighs in at over 15 MB and 111 pages. Instead of choking everyone's email server, I will share a link to the file on our DropBox account:

https://www.dropbox.com/s/aoi238renwts50v/16119.pdf?dl=0

Please look over the report and let us know if you have any questions or if you would like to see any changes.

We have all of the testing completed for the Finasol product with the exception of the two acute EC50 tests; they are going up today. The report for Finasol should go out this same time next week.

Regards,

Craig

Providing Environmental & Product Toxicity Testing since 1986

Craig Watts, Lab Director

Hydrosphere Research

11842 Research Circle

Alachua, FL 32615-6817

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To: Barron, Mace[Barron.Mace@epa.gov]; Holder, Edith[holder.edith@epa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov]; Grosser, Robert[Grosser.Robert@epa.gov]
Cc: Peter Meyer[pmeyer@hydrosphere.net]
From: Craig Watts
Sent: Tue 11/1/2016 2:27:08 PM
Subject: Sample Received

To all,

We received the third dispersant (Accell Clean DWD). We will get started on the range finding studies as soon as possible.

Craig

To: Mandsager, Kathy[kathy.mandsager@unh.edu]; Conmy, Robyn[Conmy.Robyn@epa.gov]; 'fingasmerv@shaw.ca'[fingasmerv@shaw.ca]; 'tchazen@utk.edu'[tchazen@utk.edu]; 'Robert Jones - NOAA Federal'[robert.jones@noaa.gov]; 'Samantha Joye'[mandyjoye@gmail.com]; 'ken.lee@csiro.au'[ken.lee@csiro.au]; 'mbleigh@alaska.edu'[mbleigh@alaska.edu]; 'karl.linden@colorado.edu'[karl.linden@colorado.edu]; 'kmmcfarlin@alaska.edu'[kmmcfarlin@alaska.edu]; 'thomas.s.coolbaugh@exxonmobil.com'[thomas.s.coolbaugh@exxonmobil.com]
Cc: nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Kinner, Peter[Peter.Kinner@unh.edu]; Ian P Gaudreau[ipu3@wildcats.unh.edu]
From: Lindsey R Howard
Sent: Wed 3/11/2015 7:11:25 PM
Subject: Re: FW: Dispersant Science in Arctic Waters - Degradation and Fate
Brudheim 1999.pdf
Macias-Zamora 2014.pdf

Attached are new papers.

From: Lindsey R Howard
Sent: Wednesday, March 11, 2015 2:25 PM
To: Mandsager, Kathy; 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'thomas.s.coolbaugh@exxonmobil.com'
Cc: Kinner, Nancy; Kinner, Peter; Ian P Gaudreau
Subject: Re: FW: Dispersant Science in Arctic Waters - Degradation and Fate

Attached is Mandy Joye's paper and Prince and Butler 2014.

Thanks,

Lindsey Howard

From: Mandsager, Kathy <kathy.mandsager@unh.edu>
Sent: Tuesday, March 10, 2015 4:57 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'thomas.s.coolbaugh@exxonmobil.com'
Cc: Kinner, Nancy; Kinner, Peter; Ian P Gaudreau; Lindsey R Howard
Subject: FW: FW: Dispersant Science in Arctic Waters - Degradation and Fate

This is a reminder of our WebEx meeting scheduled for tomorrow, beginning at 130pm ET. See the login instructions below. We understand that all of you are not able to participate, but we must keep plodding forward ☺

Thank you!

From: Mandsager, Kathy

Sent: Friday, February 27, 2015 3:45 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'thomas.s.coolbaugh@exxonmobil.com'
Cc: Kinner, Peter; Kinner, Nancy; Mandsager, Kathy
Subject: Dispersant Science in Arctic Waters - Degradation and Fate
Importance: High

Dear Degradation & Fate group members:

Our next meeting to discuss the outstanding items on this document, particularly with information from the older published papers (LUMCON) that address biodegradation, will be held **Wednesday, March 11 beginning at 1:30 pm ET**. Please mark your calendar and plan to participate.

Attached is the biodegradation spreadsheet for this discussion.

This meeting will be via WebEx and the instructions are noted below.

Degradation & Fate Group
Wednesday, March 11, 2015
1:30 pm | Eastern Daylight Time (New York, GMT-04:00) | 3 hrs

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Effects of Surfactant Mixtures, Including Corexit 9527, on Bacterial Oxidation of Acetate and Alkanes in Crude Oil

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Mixtures of nonionic and anionic surfactants, including Corexit 9527, were tested to determine their effects on bacterial oxidation of acetate and alkanes in crude oil by cells pregrown on these substrates. Corexit 9527 inhibited oxidation of the alkanes in crude oil by *Acinetobacter calcoaceticus* ATCC 31012, while Span 80, a Corexit 9527 constituent, markedly increased the oil oxidation rate. Another Corexit 9527 constituent, the negatively charged dioctyl sulfosuccinate (AOT), strongly reduced the oxidation rate. The combination of Span 80 and AOT increased the rate, but not as much as Span 80 alone increased it, which tentatively explained the negative effect of Corexit 9527. The results of acetate uptake and oxidation experiments indicated that the nonionic surfactants interacted with the acetate uptake system while the anionic surfactant interacted with the oxidation system of the bacteria. The overall effect of Corexit 9527 on alkane oxidation by *A. calcoaceticus* ATCC 31012 thus seems to be the sum of the independent effects of the individual surfactants in the surfactant mixture. When *Rhodococcus* sp. strain 094 was used, the alkane oxidation rate decreased to almost zero in the presence of a mixture of Tergitol 15-S-7 and AOT even though the Tergitol 15-S-7 surfactant increased the alkane oxidation rate and AOT did not affect it. This indicated that there was synergism between the two surfactants rather than an additive effect like that observed for *A. calcoaceticus* ATCC 31012.

When surfactants are applied, mixtures are often used because they perform better than the individual components (7). The exact formulations of commercial dispersants are proprietary, but the general guidelines indicate that two or more nonionic surfactants with different water solubilities and one or more charged surfactants, preferably anionic, are used and that all of the compounds are dissolved in a solvent consisting of water, water-miscible hydroxy compounds, or hydrocarbons (5). Corexit 9527, a frequently mentioned oil spill dispersant, was developed for use on open sea oil slicks. This dispersant is composed of about 48% nonionic surfactants, including ethoxylated sorbitan mono- and trioleates (Tween 80 and Tween 85) and sorbitan monooleate (Span 80), about 35% anionic surfactants, including sodium dioctyl sulfosuccinate (AOT), and about 17% ethylene glycol monobutyl ether as a solvent (13). There have been reports of both negative and positive effects of Corexit 9527 on bacterial degradation of crude oil (6, 11, 14). The explanations given for the effect of this surfactant mixture vary from a negative effect on the hydrocarbon uptake rate to a positive effect due to increased surface area of the substrate (12).

In recent reports there has been a strong emphasis on studying surfactant-bacterial cell interactions to determine the influence of surfactants on alkane oxidation (2±4).In the present study, we compared surfactant mixtures like oil spill dispersant mixtures with the individual components of the mixtures. The effects of the surfactants on acetate oxidation rates and uptake rates were also investigated since the results could provide information about how the individual surfactants and mixtures of surfactants affect cell processes. This was important since in previous work (2, 4) researchers focused on the physicochemical functions of the surfactants; in this study we examined the interactions of the surfactants with bacterial cells.

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MATERIALS AND METHODS

Bacterial isolates. *Rhodococcus* sp. strain 094 was obtained from the FINA Culture Collection kept at SINTEF Applied Chemistry, Group of Biotechnology, Trondheim, Norway. This isolate was obtained from enrichment cultures by using inocula from Norwegian coastal waters and was an alkane oxidation-positive organism (1, 9). *Acinetobacter calcoaceticus* ATCC 31012 was purchased from the American Type Culture Collection (Rockville, Md.). Suspensions of oil-grown and acetate-grown bacteria in 15% glycerol were stored in 1-ml cryotubes at 2 80°C.

Media. The seawater medium used has been described previously (2). The concentration of crude oil or acetate was 0.5%.

Compounds. Tergitol 15-S-3 ($C_{11-15}E_3$, HLB 8.0), Tergitol 15-S-7 ($C_{11-15}E_7$, HLB 12.1), Tergitol 15-S-15 ($C_{11-15}E_{15}$, HLB 115.4), and Tergitol 15-S-30 ($C_{11-15}E_{30}$, HLB 20.6) are polyglycolether surfactants. Span 20 (HLB 8.6) and Span 80 (HLB 4.3) are laureate and stearate sorbitan fatty acid esters, respectively. Tween 85 (HLB 11.0) is an $(ethoxy)_{20}$ sorbitan trioleate ester, while Tween 80 (HLB 15.6) is the monooleate ester. The Tergitol, Span, Tween, AOT, and sodium dodecyl sulfate surfactants were purchased from Sigma Chemical Co., St. Louis, Mo. Corexit 9527 was kindly provided by P. J. Brandvik, SINTEF, Trondheim, Norway. [1-^{14}C]hexadecane and [2-^{14}C]acetate were purchased from Amersham, Little Chalfont, United Kingdom. The medium constituents were obtained from Merck, Darmstadt, Germany.

Oxidation rate measurement. The protocol which we used to measure oxidation rates has been described previously (2). Oxidation rates (in microliters of O_2 per hour per milligram [dry weight]) were determined by Warburg respirometry. The cells were pregrown for 48 h (to the early stationary phase) in 500-ml shake flasks containing 100 ml of medium at 25°C, centrifuged at 15,000 3 *g*, and washed twice in N-free mineral medium. A 150-ml portion of each cell suspension (5 to 10 mg [dry weight]/ml) was transferred to the side arm of a Warburg flask (20 ml). The standard concentrations used were 0.5% (wt/vol) oil and 0.01% (wt/vol) surfactant. Surfactant-treated oil and N-free mineral medium (1 ml) were premixed in the central compartment during 30 min of temperature equilibration (25°C) before the cells were added. In some experiments the crude oil was replaced with 10 mM acetate as the substrate. Mineralization of acetate and alkanes was assessed by determining the amount of $^{14}CO_2$ produced from [2-^{14}C]acetate (150,000 dpm/flask) or from [1-^{14}C]hexadecane (50,000 dpm/flask) present in the oil. The contents of the CO_2 trap (0.1 ml of 2 M NaOH) in the center well were transferred to Opti-Fluor scintillation cocktail (Packard) and counted with a Wallac model s1410 scintillation counter. Every experiment was performed at least twice with three flasks for every condition. The results of one representative experiment are presented below, and the statistical variations are indicated by the standard deviations.

TABLE 1. Effects of Corexit 9527, four of its component surfactants, and one mixture on crude oil oxidation by oil-grown *A. calcoaceticus* ATCC 31012

Prepn	Oxidation rate (ml of O_2/h/mg [dry wt])[a]	$^{14}CO_2$ radioactivity (dpm)[b]
Oil	16.7 6 1.4	400 6 50
Oil 1 Corexit 9527 (0.01%)	9.7 6 0.5	250 6 150
Oil 1 Span 80 (0.01%)	41.1 6 1.0	1,800 6 200
Oil 1 Tween 85 (0.01%)	19.0 6 2.4	700 6 250
Oil 1 Tween 80 (0.01%)	16.0 6 0.7	300 6 200
Oil 1 AOT (0.005%)	6.1 6 2.5	
Oil 1 Span 80 (0.01%) 1 AOT (0.005%)	30.3 6 0.9	

[a] Oxidation in the presence of 0.5% (wt/vol) crude oil and 0.01 or 0.005% (wt/vol) surfactant in artificial seawater without nitrogen. The endogenous respiration rate with oil was 3.0 6 0.3 ml of O_2/h/mg (dry weight).

[b] Amount of $^{14}CO_2$ recovered from the NaOH trap at the end of the experiment.

TABLE 2. Effects of Corexit 9527 and four surfactants on acetate oxidation by oil-grown *A. calcoaceticus* ATCC 31012 cells in the stationary phase of growth

Prepn	Oxidation rate (ml of O_2/h/mg [dry wt])[a]	$^{14}CO_2$ radioactivity (dpm)
Acetate	5.0 6 0.3	2,300 6 500
Acetate 1 Span 20 (0.01%)	32.3 6 1.7	14,950 6 2,500
Acetate 1 Tween 85 (0.01%)	31.0 6 3.0	14,700 6 550
Acetate 1 Tween 80 (0.01%)	26.7 6 2.7	14,900 6 350
Acetate 1 Corexit 9527 (0.01%)	25.9 6 3.2	15,350 6 550
Acetate 1 AOT (0.005%)	0.5 6 1.7	
Acetate 1 Span 20 (0.01%) 1 AOT (0.005%)	15.3 6 1.7	

[a] The endogenous respiration rate with acetate was 2.0 6 0.7 ml of O_2/h/mg (dry weight).

[^{14}C]acetate uptake. Cells were pregrown and washed cell suspensions were prepared as described above for the oxidation studies. Twenty milliliters of a cell suspension (5 to 10 mg [dry weight]/ml) containing surfactants was mixed with 10 mM [^{14}C]acetate (150,000 dpm/ml). After 5 and 15 min three 2-ml aliquots were removed and filtered with a type GF/F 47-mm-diameter Whatman microfiber filter. The filters were washed with 10 ml of mineral medium and transferred to scintillation vials containing 10 ml of Hisafe III scintillation fluid (Pharmacia). After 2 h of equilibration, the radioactivity was measured with the Wallac model s1410 scintillation counter. Heat-inactivated *Rhodococcus* sp. strain 094 cells were warmed to 100°C and cooled rapidly to room temperature in a water bath. Viable counting indicated that less than 0.5% of the cells survived.

RESULTS AND DISCUSSION

***A. calcoaceticus* ATCC 31012.** The oxidation rates of *A. calcoaceticus* ATCC 31012 were determined by Warburg respirometry as described previously (2). These rates were corrected for O_2 uptake by using cell suspensions containing surfactants but no crude oil. In each case the presence of surfactants resulted in a small increase in the respiration rate, but this increase did not exceed two times the endogenous respiration rate.

Corexit 9527 decreased the rate of oxidation of alkanes in crude oil by *A. calcoaceticus* ATCC 31012 rather strongly (Table 1). On the other hand, sorbitan monooleate (Span 80, a Corexit 9527 constituent) increased the oxidation rate very markedly. Tween 85 and Tween 80, the two other surfactant components of Corexit 9527, did not affect and slightly increased the oil oxidation rate, respectively. AOT, the prominent anionic surfactant constituent of Corexit 9527, had a very strong negative effect on the oil oxidation rate. The combination of Span 80 and AOT increased the oxidation rate, but not as much as Span 80 alone increased it. The correlation between Corexit 9527 and the mixture containing Span 80 and AOT was not quantitatively substantiated, but this may have been due to differences in surfactant concentrations and the presence of Tween 80, Tween 85, and other anionic surfactants in Corexit 9527. The mineralization data, expressed as endpoint values for the amount of $^{14}CO_2$ that evolved from [1-^{14}C]hexadecane-spiked oil, validated the oxidation results. The solvent of Corexit 9527, ethylene glycol monobutyl ether, had no effect on the oxidation rate (data not shown).

In experiments performed with acetate as the substrate Span 80 was replaced by Span 20 due to the very poor water solubility of the former compound. Span 20 had the same positive effect on the oil oxidation rate that Span 80 had (4). Oil-grown *A. calcoaceticus* ATCC 31012 cells had a very low specific oxidation rate for acetate (Table 2). In the presence of Span 20 the oxidation rate increased almost six times. This was not due to oxidation of Span 20 but was due to increased oxidation of acetate, as confirmed by $^{14}CO_2$ recovery data obtained with [2-^{14}C]acetate. The other sorbitan surfactants and Corexit 9527 also increased the rate of oxidation of acetate to the same degree, in contrast to the situation for oil oxidation, where only Span 20 increased the oxidation rate. Furthermore, the negatively charged surfactant AOT drastically decreased the acetate oxidation rate, and the positive effect of the Span 20-AOT mixture was much less than the positive effect of Span 20 alone. Span 20-AOT mixtures thus had very similar effects on the oxidation of alkanes and the oxidation of acetate in *A. calcoaceticus* ATCC 31012.

The acetate oxidation data were correlated with acetate uptake rates. The uptake of [2-^{14}C]acetate increased significantly in the presence of the nonionic surfactants and Corexit 9527 (Table 3). AOT had very little effect on the rate of uptake of acetate. Therefore, AOT had a strong negative effect on oxidation of acetate but not on transport of acetate, while the nonionic surfactants and Corexit 9527 increased the rate of acetate oxidation, probably by increasing the transport rates. AOT did not influence the effect of Span 20 on the acetate uptake rate, which contrasts with the effect of the mixture on both the alkane and acetate oxidation rates. It seems, therefore, that the effect of the surfactant mixture on acetate oxidation was the sum of two independent effects, the effect of AOT on the oxidation machinery (a negative effect) and the effect of Span 20 or Span 80 on the transport machinery (a positive effect).

Only two of the nonionic surfactants examined, Span 20 and Span 80, increased the alkane oxidation rates. This indicates that the effects of the nonionic surfactants on the alkane oxidation rate were not due to the general amphiphilic properties of the surfactants but rather to a specific interaction determined by both the chemical structures and the physicochemical properties of the surfactants. In addition, the effects of the surfactants are also probably determined in part by the structure of the components in the bacterial cell envelope. Based on these findings and the acetate uptake and oxidation results, it may be hypothesized that the overall effect of Corexit 9527 on alkane oxidation, as well as acetate oxidation, is the sum of independent effects exerted by the individual surfactants in the surfactant mixture.

***Rhodococcus* sp. strain 094.** The mixture containing Span 20 and AOT and the individual surfactants were also tested with the gram-positive organism *Rhodococcus* sp. strain 094. Span 20 slightly increased the alkane oxidation rate, while AOT and

TABLE 3. Effects of Corexit 9527 and four surfactants on [2-^{14}C]acetate uptake by oil-grown *A. calcoaceticus* ATCC 31012

Prepn	[2-^{14}C]acetate uptake (dpm)	
	5 min	15 min
Acetate	2,100 6 100	4,500 6 400
Acetate 1 Span 20 (0.01%)	5,700 6 500	33,000 6 1,000
Acetate 1 Tween 85 (0.01%)	5,000 6 750	41,000 6 2,500
Acetate 1 Tween 80 (0.01%)	3,500 6 1,300	23,900 6 500
Acetate 1 Corexit 9527 (0.01%)	5,000 6 1,200	33,000 6 1,500
Acetate 1 AOT (0.005%)	1,850 6 50	4,500 6 50
Acetate 1 Span 20 (0.01%) 1 AOT (0.005%)	5,300 6 300	35,100 6 700

the mixture containing the two surfactants had little or no effect on the oxidation rate (Table 4, experiment A). Span 20 was replaced by Tergitol 15-S-7, which is known to increase the alkane oxidation rate in *Rhodococcus* sp. strain 094 (2). Tergitol 15-S-7 caused a threefold increase in the oil oxidation rate in oil-grown cells (Table 4, experiment B). AOT alone slightly increased the oxidation rate. Mixing the two surfactants, however, resulted in almost complete cessation of alkane oxidation. The endogenous respiration of the cells in the presence of the surfactant mixture was also severely reduced (data not shown). Tergitol 15-S-7 interacted strongly with *Rhodococcus* sp. strain 094 cells since it strongly increased the oil oxidation rate. In a mixture with AOT, Tergitol 15-S-7 may decrease the expected repulsion between the negatively charged bacterial cells and the negatively charged compound AOT. This may give AOT access to structures in the cell envelope that are not available to AOT alone and thus may explain the observed synergistic effect. Span 20 did not influence the positive effect of Tergitol 15-S-7 (Table 4, experiment C), which may indicate that Span 20 interacts much more weakly than Tergitol 15-S-7 with cell structures. Therefore, as shown in Table 4 (experiment A), Span 20 cannot facilitate AOT's access to cell structures that are critical for the integrity of the cells. This may also explain the observed effects of the homologous Tergitol compounds shown in Table 4 (experiment D). The two more hydrophobic surfactants, Tergitol 15-S-7 and Tergitol 15-S-3, increased the rate of alkane oxidation by *Rhodococcus* sp. strain 094 cells grown from the stationary phase (2). The strong interactions between the surfactants and the cells resulted in almost complete cessation of alkane oxidation when the two surfactants were mixed with AOT (Table 4, experiment D). The two more hydrophilic surfactants, Tergitol 15-S-15 and Tergitol 15-S-30, did not significantly increase the rate of alkane oxidation by *Rhodococcus* sp. strain 094 cells grown from the stationary phase (2). When Tergitol 15-S-15 and Tergitol 15-S-30 were mixed with AOT, the decreases in the oxidation rate were much less than the decreases observed with Tergitol 15-S-3 and Tergitol 15-S-7, in accordance with the weaker interactions of the former nonionic surfactants with the cells.

Separately, Tergitol 15-S-7 and AOT decreased the rate of oxidation of acetate by 30 to 40%, whereas a mixture containing both of these compounds decreased the oxidation rate to almost zero (Table 4, experiment E). The rates of uptake of [2-^{14}C]acetate by *Rhodococcus* sp. strain 094 cells (Table 5) in the presence of Tergitol 15-S-7 or AOT were positively correlated with the acetate oxidation data shown in Table 4 (experiment E). Tergitol 15-S-7 and AOT separately affected acetate oxidation by reducing the specific transport of acetate. The Tergitol 15-S-7 ± AOT mixture resulted in uptake of acetate corresponding to the uptake by heat-inactivated cells.

TABLE 4. Effects of surfactant mixtures on crude oil and acetate oxidation by oil-grown *Rhodococcus* sp. strain 094[a]

Expt	Prepn	Oxidation rate (ml of O_2/h/mg [dry wt])
A[b]	Oil	5.1 6 0.3
	Oil 1 Span 20 (0.01%)	6.1 6 0.1
	Oil 1 AOT (0.005%)	5.5 6 0.4
	Oil 1 Span 20 1 AOT	5.8 6 0.1
B[c]	Oil	3.5 6 0.4
	Oil 1 Tergitol 15-S-7 (0.01%)	10.9 6 0.5
	Oil 1 AOT (0.005%)	5.0 6 0.1
	Oil 1 Tergitol 15-S-7 1 AOT	0.3 6 0.2
C[d]	Oil	3.4 6 0.1
	Oil 1 Tergitol 15-S-7 (0.01%)	8.4 6 0.5
	Oil 1 Span 20 (0.005%)	5.0 6 1.3
	Oil 1 Tergitol 15-S-7 1 Span 20	8.4 6 0.9
D[e]	Oil	6.2 6 0.5
	Oil 1 Tergitol 15-S-7 (0.01%)	9.5 6 0.2
	Oil 1 AOT (0.005%)	7.0 6 0.3
	Oil 1 Tergitol 15-S-7 1 AOT	1.1 6 0.2
	Oil 1 Tergitol 15-S-3 1 AOT	1.2 6 0.2
	Oil 1 Tergitol 15-S-15 1 AOT	4.2 6 2.0
	Oil 1 Tergitol 15-S-30 1 Span 20	5.2 6 0.9
E[f]	Acetate	21.5 6 1.4
	Acetate 1 Tergitol 15-S-7 (0.01%)	15.5 6 1.0
	Acetate 1 AOT (0.005%)	13.6 6 2.0
	Acetate 1 Tergitol 15-S-7 1 AOT	0.5 6 0.6

[a] For experimental conditions see Table 1.
[b] The endogenous respiration rate with oil was 2.4 6 0.1 ml of O_2/h/mg (dry weight).
[c] The endogenous respiration rate with oil was 1.5 6 0.2 ml of O_2/h/mg (dry weight).
[d] The endogenous respiration rate with oil was 1.3 6 0.1 ml of O_2/h/mg (dry weight).
[e] The endogenous respiration rate with oil was 2.1 6 0.3 ml of O_2/h/mg (dry weight).
[f] The endogenous respiration rate with acetate was 1.9 6 0.4 ml of O_2/h/mg (dry weight).

Comparison of acetate- and oil-grown *A. calcoaceticus* ATCC 31012 and *Rhodococcus* sp. strain 094. The alkane oxidation genes are not constitutively expressed in most gram-positive and gram-negative bacteria. There is a necessary induction period prior to growth on alkanes, and there is derepression of the alkane oxidation system, as well as a system for uptake of and adhesion to the hydrophobic substrate (8). The latter very often coincides with synthesis of biosurfactants, which alter the cell surface topology of the degrading cells (10). *Rhodococcus* sp. strain 094 gains a hydrophobic surface and adheres to the hexadecane phase when it is transferred from acetate-containing medium to hexadecane-containing medium (1). *A. calcoaceticus* Rag-1 generally is very hydrophobic during growth on hexadecane and produces a water-bound heteropolysaccharide bioemulsifier named emulsan. A comparative study of the effects of surfactants on acetate- and oil-grown cells might provide information about the dissimilarities of these two types of cells.

Oil-grown *A. calcoaceticus* ATCC 31012 cells had a very low specific activity for acetate oxidation compared to acetate-grown cells (5 versus 42 ml of O_2/h mg [dry weight]^{21}) (Table 2; data not shown). In the presence of Corexit 9527, Span 20, Tween 80, and Tween 85 the specific rate of acetate oxidation in oil-grown cells increased to approximately the rate in ace-

TABLE 5. Effects of two surfactants and mixtures on the uptake of [2-^{14}C]acetate by oil-grown *Rhodococcus* sp. strain 094

Prepn	[2-^{14}C]acetate uptake (dpm)	
	5 min	15 min
Acetate	16,800 6 900	47,200 6 600
Acetate 1 Tergitol 15-S-7 (0.01%)	9,250 6 1,200	18,500 6 400
Acetate 1 AOT (0.01%)	9,900 6 50	18,600 6 700
Acetate 1 Tergitol 1 AOT	900 6 150	1,450 6 100
Acetate 1 heat-inactivated cells	800 6 250	1,100 6 100

tate-grown cells. This indicated that there was surface restriction of acetate transport that was circumvented by the added surfactants. AOT affected acetate oxidation and alkane oxidation in the same negative way in oil-grown cells, and the presence of an interacting nonionic surfactant partially counteracted the action of AOT (Tables 1 and 2). This may indicate that overall oxidation of acetate in oil-grown cells of *A. calcoaceticus* ATCC 31012 is restricted by the speci®c surface conditions of cells induced to grow on hydrophobic substrates.

In acetate-grown cells of *A. calcoaceticus* ATCC 31012, Span 20 caused a moderate (10%) decrease in the acetate oxidation rate, and AOT and the mixture of the two compounds decreased the acetate oxidation rate by 20% (data not shown), in sharp contrast to the results obtained for the oil-grown cells. These ®ndings illustrate the marked difference between oil-grown and acetate-grown cells of this gram-negative bacterium, which most likely is linked to differences in surface structure or topography.

A mixture of Tergitol 15-S-7 and AOT affected acetate oxidation in acetate-grown cells of *Rhodococcus* sp. strain 094 in the same way (data not shown) that it affected acetate oxidation in oil-grown cells (Table 4, experiment E); however, when tested separately, the surfactants had no effect on acetate-grown cells, in contrast to the marked negative effect that they had on oil-grown cells. While Tergitol 15-S-7 markedly affected acetate oxidation in oil-grown cells, the results suggest that there was only a weak interaction in acetate-grown cells, which clearly indicated that there are structural differences between the two types of cells. The weak interaction of Tergitol 15-S-7 with acetate-grown cells was, however, suf®cient for the dramatic negative synergistic effect with AOT to take place.

In summary, we found that the effects of surfactant mixtures on bacterial metabolism may not always be easily predicted on the basis of the effects of the individual surfactants in the mixtures. Admittedly, our information is limited, but two main conclusions appear to be relevant. The surfactants in a mixture may independently affect various sites in the cell and have an overall effect which is additive. This seems to be case for *A. calcoaceticus* ATCC 31012. Alternatively, surfactants may in¯uence each other's interactions with cells, resulting in synergistic effects. This seems to be the case for the gram-positive organism *Rhodococcus* sp. strain 094.

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On the effects of the dispersant Corexit 9500© during the degradation process of n-alkanes and PAHs in marine sediments

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Abstract In many coastal countries, oil spill contingency plans include several alternatives for removal of the spilled oil from the ocean. Frequently, these plans include dispersants. Because this process applies chemical substances that may add toxicity to oil that already contains toxic compounds, it is, at times, a controversial method to fight oil pollution. Additionally, local conditions may result in particular complications. We investigated the possible effects of the dispersant Corexit 9500© under conditions similar to those of subtropical oceans. We used fuel oil #6+ diesel as the test mixture. Under certain conditions, at least part of the dispersed oil may reach the sediment, particularly if the dispersant is applied in coastal waters. Nine experimental units were used in this experiment. Similar conditions of water temperature, salinity, air fluxes into the experimental units, and hydrocarbon concentrations in sediments were used. Two treatments and one control, each one with three replicates, were carried out. We concentrated our investigation on sediment, although measurements of water were also taken. Our results suggest that once the oil has penetrated the sediment, no significant differences exist between oil that contains dispersant and oil without dispersant. Noticeable degradation of aliphatic hydrocarbons occurred mainly in the low molecular weight aliphatic hydrocarbons and not in the others. Apparently, degradation of aromatics was easier than that of alkanes. However, some differences were noticed for the degradation of PAHs in the sediment, suggesting a faster degradation under particular conditions in aerobic environments such as under this experiment.

Keywords Oil dispersants · Corexit 9500© · Oiled marine sediments · PAHs · n-Alkanes

Introduction

Oil is one of the most prevalent forms of pollution in the marine environment (Martínez-Alonso and Gaju 2005; Vila et al. 2010). Both crude oil and its derivatives are very complex mixtures containing hundreds and even thousands of different compounds. As such, once a significant oil spill or a spill of oil subproducts is released into the ocean, procedures are often implemented by governments and other organizations to protect ocean resources. One of these procedures relies on the employment of chemical substances known as dispersants. Dispersants are designed to remove the oil from the surface of the ocean; in the process, the oil becomes dispersed in the water column (Kujawinski et al. 2011). The principle involved in the use of dispersants has been explained elsewhere (see, for example, Lewis and Daling 2001). In any case, the oil becomes dispersed by the use of surfactants, and the

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small droplets generated are incorporated into the water column. Once in the water column, these droplets may be colonized by bacteria and degrade, but they can become attached to particles and be removed to the bottom sediment, particularly in coastal waters where biogenic and inorganic particles are abundant.

The use of dispersants such as the commercial product Corexit 9500© is still controversial, and the results of different experiments have been inconclusive with respect to possible added toxicity or combined toxicity (oil + dispersant), as well as with respect to its potential effects on the rates of degradation of hydrocarbons (Leahy and Colwell 1990; Ramachandran et al. 2004).

Dispersants, such as Corexit 9500©, are often made up of three main components: a solvent, an additive, and a surface-active compound. The solvents are used to promote dissolution of the ingredients, and the surface-active compounds reduce the surface tension of molecules so that mixing can take place, improving the solubility of the oil components. Surfactants have both a lipophilic and a hydrophilic part. Their third component includes additives that stabilize the formula. Once dispersed, the small oil droplets may become attached to particles and particulate materials and eventually reach the sediments (National Research Council 2005).

The effect of surfactants on the mixture and on the hydrocarbons involves complex interactions between oil, surfactant, water, and the microbial community. These interactions are affected by several variables including the viscosity of the particular oil (Ross 2006), as well as water temperature and salinity, among others (Bruheim et al. 1997).

Several studies have included sediment analysis after oil spills (Hoffman and Quinn 1979) in controlled ecosystems (Gearing et al. 1980; Wade and Quinn 1980; Gearing and Gearing 1982a, b). Mackay and Hussain (1982) have also studied the physicochemical interaction of oil droplets with particulate material. Payne et al. (2003) have suggested that the interaction with particles is important for the transport of hydrocarbons to the sediment. Size increases due to agglomeration or biological packing favors sedimentation. In addition, McNaughton et al. (2003) studied the effect of Corexit 9500© on the dispersion, biodegradation, and microbial colonization of two types of crude oil at 8 and 15 °C. Otiloloju (2005) worked on the toxic effects of dispersant alone as compared to spent oil in an effort to obtain adequate organisms to be used as sentinels for oil pollution and dispersants in fragile ecosystems.

There are, however, many concerns about the potential effects of the dispersant once it is mixed with crude or with a specific oil subproduct. There are also questions about dispersants' applicability under different ecological conditions and different biota. In fact, there has been an emphasis on studying the effects of dispersants and oil in water columns (Mulkins-Phillips and Stewart 1974; Lindstrom and Braddock 2002; McNaughton et al. 2003; Otiloloju 2005; Venosa and Holder 2007), but less research appears to have been directed to sediments as a potential final destination of most mixtures of oil and detergent.

We decide to investigate the possible effects of the dispersant Corexit 9500© under conditions similar to those of subtropical oceans and using Mexican oil products. Our goal was to determine if the presence of these surfactant chemicals would help accelerate or, on the contrary, diminish the degradation of this particular Mexican formulation once it is mixed with oil. Although one particular formulation was used, this study is applicable to other oil mixtures of similar formulations.

The contingency plans in the case of spills in Mexican coastal waters contemplate the use of these substances, and we wanted to contribute relevant information on the potential of these commercial formulas to work under subtropical conditions. In most protection plans from different countries, a series of considerations are taken into account before the use of these substances becomes advisable (Lindstrom and Braddock 2002).

The purpose of this work was to determine what the effect would be, if any, in the presence of the commercial dispersant Corexit 9500© on the degradation process for n-alkanes and PAHs when these mixtures are combined in seawater and, in particular, in sediments at typical subtropical temperatures and salinities. The hydrocarbon mixture was obtained from a local plant of the Mexican oil company PEMEX.

Methods

To obtain the seed for the inoculation with bacteria capable of degrading oil, we selected two sites: Rosarito, Baja California (B.C.) and Todos Santos Bay, B.C. At Rosarito B.C. (Fig. 1), there is a discharging conduit to the storage area of PEMEX (the

Mexican oil company) on the coast where this fuel is used for electricity generation.

We used a Van Veen grab to collect sediment from both places. The sediment was placed in a container and cooled (in ice), and then transported to the laboratory for its use.

The fuel mixture used in this experiment was an oil subproduct design under the name IFO-15. This mixture contains 85 % fuel oil (#6) and 15 % diesel. It is used on ships and also as a flow stopper as the first material drained from ducts during discharging maneuvers to storage tanks.

All glassware used for extraction and sample concentrations was previously washed with diluted soap (Micro®) at 2 %. Once rinsed with clean water and dried, the material was set in a furnace at 400 °C for 4 h.

Grain size

For grain size determination, we used a Horiba LA-920 Grain Size Analyzer (Horiba Instruments, Irvine, CA, USA) laser diffraction analyzer with a laser diffraction–scattering method. A pretreated sample was introduced into the analyzer with ~300 cm^3 of 0.2 % sodium pyrophosphate solution, which aided in the dispersion. Calibration of the instrument included the use of a standard from USGS.

Organic carbon

Organic C (OC) was measured with an elemental analyzer (model 1106, Carlo Erba, Milan, Italy) connected to a Minigrator integrator (Spectra Physics, Santa Clara, CA, USA). Before the OC was determined, we eliminated the carbonates from each sample by soaking it in 0.1 M HCl for 24 h. A calibration standard was also utilized (MAG, with a nominal average value of 2.15 % (and 0.02 SD). Although both grain size and OC were measured, given the homogenization of the samples, no effects were expected from these parameters under the limited number of samples used for this experiment.

Experimental design

The design consisted of nine containers, all under similar conditions of water temperature, air (provided

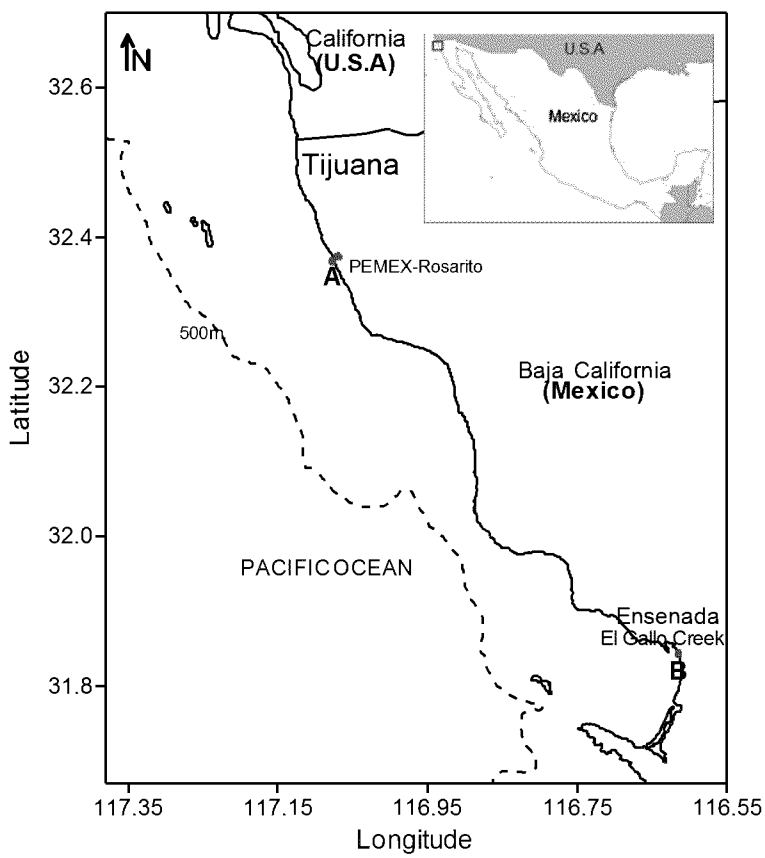

Fig. 1 Sites A and B are the places at Rosarito, B.C. and the discharge site for El Gallo Creek, respectively, where the sediment seeds were collected

from similar air pumps), and hydrocarbon concentration. The experiment included two treatments and one control (C), and each was made up of three replicates (Table 1).

The experimental units were randomized to reduced experimental error and avoid demonic intrusions (Hurlbert 1984).

Experimental units

Preparation

The collected sediments had to be partially dried to allow for the incorporation of the fuel mixtures. Wet sediment is not appropriate for the addition of hydrocarbons. To avoid potential alteration of their composition, the sediments were dried at very low temperatures (30 °C). Once dried, the fuel was weighed and evenly distributed in the nine experimental units.

To incorporate the hydrocarbon mixture, a solution was prepared using 15 g of IFO-15 dissolved in dichloromethane (DCM) and made up to 500 ml in pentane. From that stock solution, we took 5 ml and diluted it to a final 500 ml in pentane. To help homogenize the sample and evaporate the excess solvent, all treatments were placed in an agitation bath. Once the solvent was evaporated, wet sediment was incorporated in the weights and proportions shown in Table 1.

A similar procedure was followed for treatment 2, except for the addition of the dispersant Corexit 9500© following the indications of McNaughton et al. (2003). These authors suggested the incorporation of the dispersant as 10 % of the total weight of the hydrocarbons. Pentane was the solvent used for the incorporation of the mixtures. Once the solvent was incorporated and volatilized, wet sediment was added in accordance with the procedure for treatment 1.

After 36 h of rest, 5 l of filtered seawater was added to each experimental unit. The units were placed according to the randomization procedure and were equipped with an aeration unit to avoid hypoxic or even anoxic conditions in the sediment. All experimental units were set to the same water temperature of 16.0±0.2 °C, which was kept constant during the 5-month-long experiment.

The experiment began on June 21, 2007. Sampling was carried out in a biweekly manner. At those times, samples from the water and from the sediment were collected and processed to analyze for the presence of both chemical groups. For sediments, 1 g was analyzed each time. For water, we used 50-ml samples.

Instrumental methods, extraction, and cleanup

The method used was that of Zeng and Vista (1997). For sediments, we used the Soxhlet extraction method. Briefly, 15 g of dried sediment was extracted for 12 h using 150 ml of DCM; elemental sulfur was removed by means of an activated copper wire to avoid interferences.

For water samples, each sample was extracted using a separatory funnel. We took 50-ml water samples that were extracted with three portions of DCM (25 ml each time). All three extractions were combined into one.

All extracts (from sediments or from water) were concentrated using a water bath with Snyder columns. The extracts were recovered with hexane and concentrated to 1 ml under a nitrogen flow. Each extract was further cleaned up by column chromatography with silica/alumina-packed columns. Hexane (15 ml) was used to extract the n-alkanes (F1). The polyaromatic hydrocarbons were extracted from the samples using 40 ml of a mixture of dichloromethane/hexane (30:70, v/v) (F2). All fractions were concentrated down to 1.0 ml and placed in amber vials with Teflon liners for GC-FID or GC-MS analysis.

All sample extracts (F1 and F2) were analyzed using a gas chromatograph (Hewlett-Packard model 6890) with a flame ionization detector (FID). Briefly, the conditions were as follows: 1 µl was injected in the splitless mode. The injection port temperature was 280 °C, and the detector port temperature was 300 °C. The initial oven temperature was programmed at 70 °C

Table 1 Experimental design used for the degradation of the Mexican mixture IFO-15 under the effects of the dispersant in marine sediments

Treatment 1 (CIFO)	Treatment 2 (DIFO)	Control (C)	Replicates per treatment
Sediment contaminated with IFO-15	Sediment contaminated with IFO-15+ dispersant	Sediment only	3

Table 2 Priority polyaromatic hydrocarbons and their quantification ion

Quantification ion	Compound
128	Naphthalene
152	Acenaphthene
154	Acenaphthylene
166	Fluorene
178	Phenanthrene
178	Anthracene
202	Fluoranthene
202	Pyrene
228	Benzo(a)anthracene
228	Chrysene
252	Benzo(b)fluoranthene
252	Benzo(k)fluoranthene
252	Benzo(a)pyrene
276	Indene(1,2,3-cd)pyrene
276	Benzo(g,h,i)perylene
278	Dibenzo(a,h)anthracene

(for 5 min), increasing to 300 °C at a rate of 5 °C/min. The final temperature was maintained for 28 min. The mobile phase was He at a constant pressure of 8.83 psi. We used a capillary column HP-5 (5 % methyl-phenyl polysiloxane) of 30 m long with an id of 250 μm and a stationary phase thickness of 0.25 μm.

A fortified control for standards was used for both aromatic and alkyl hydrocarbons to calculate percentage recoveries from sample extracts. This was done for both water and sediment samples. The analytical controls also included procedural blanks, fortified controls, and external calibration containing seven concentration levels using commercial standards. In addition, internal standards were also used.

priority polyaromatic hydrocarbons: naphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indene(1,2,3-cd)pyrene, and benzo(g,h,i)perylene, and Dibenzo (a,h) anthracene. In Table 2, we show the quantification ions used.

Calculations

To determine possible significant differences between the average initial (15 days) and final (120 days) concentrations for each treatment, Student's t tests were carried out for independent samples (95 % confidence). We divided the n-alkanes into two groups: light (C_{14} to C_{18}, pristane, and phytane; n=7) and heavy (C_{19}–C_{36}; n=17). However, the aromatic hydrocarbons were considered as a whole (n=6). Tests for normality (Kolmogorov–Smirnov and Lilliefors) as well as homoscedasticity were conducted for the data (F test for two samples). When the assumptions of homoscedasticity

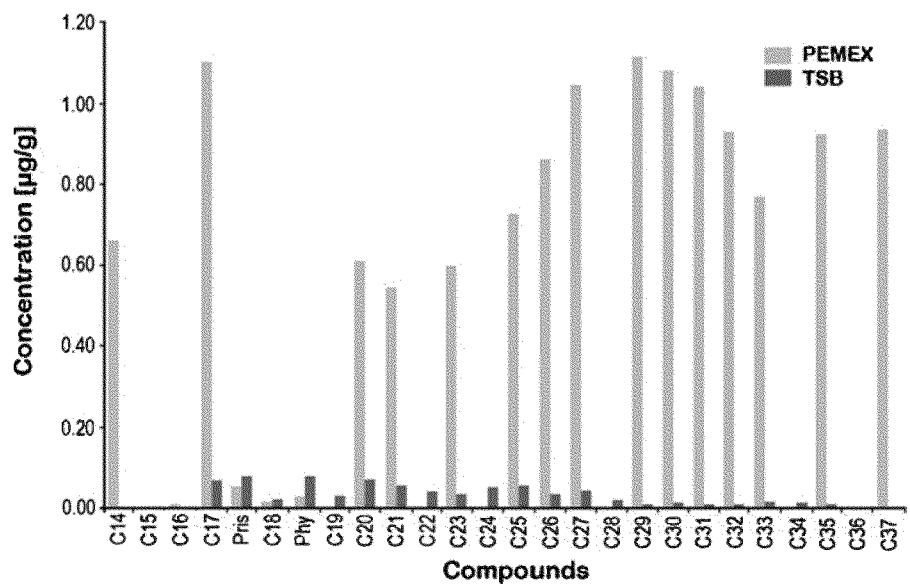

Fig. 2 Aliphatic hydrocarbons found in the seed sediments collected from the PEMEX discharging site in Rosarito, B. C. and in the sediments at Todos Santos Bay (TSB) at the old discharge zone for the wastewater treatment plant

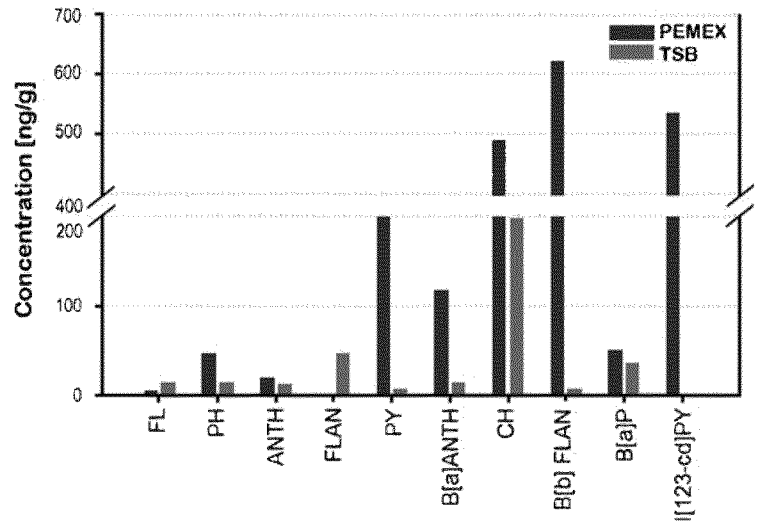

Fig. 3 PAHs measured in the seed sediments collected from the downloading zone at PEMEX in Rosarito, B.C. and Todos Santos Bay (TSB) in Ensenada, Mexico. From left to right, the compounds are as follows: FL fluorene, PH phenanthrene, ANTH anthracene, FLAN fluoranthene, PY pyrene, B[a]ANTH benzo[a]anthracene, CH chrysene, B[b]FLAN benzo[b]fluoranthene, B[a]P benzo[a]pyrene, I[123-cd]PY indene[1,2,3-cd]pyrene

and normality were not met, a non-parametric test—the Wilcoxon test—for paired samples was used.

The differences between treatments (oil without dispersant vs. oil and dispersant) were carried out using Student's t tests for independent samples (95 % confidence, n=6). All tests were carried out using Statistica 7 and Excel 2007.

Results and discussions

The sediments used as seed originally contained both n-alkanes, which are shown in Fig. 2, as well as aromatic hydrocarbons, shown in Fig. 3. The sediments from Rosarito, B.C. (near the PEMEX installation) contained several times more hydrocarbons than those from Todos Santos Bay (TSB in the figures).

The percentage recoveries that were obtained for both the aromatic and aliphatic fractions are listed in Table 3.

The limit of detection (LOD) for these compounds was calculated by the methods proposed by Foley and Dorsey (1984) and Vial and Jardy (1999). The values ranged from 0.003 to 0.19 μg/g (average of 0.08 μg/g) for n-hydrocarbons and from 0.026 to 0.15 ng/g (average of 0.085 ng/g) for PAHs.

Some PAHs were also present in the sediments used to prepare the experimental units, and they are shown in Fig. 3; moreover, more PAHs were detected in the sediments from Rosarito than in those from TSB. The largest concentrations found were for four compounds (benzo(b)fluoranthene, indene(1,2,3-cd)pyrene, chrysene, and pyrene).

Oil characterization

We also identified all the aliphatic hydrocarbons in the original oil mixture. The typical chromatogram for the oil labeled as IFO-15 contained n-alkanes from C_{14} to C_{40}. The characteristic isoprenoids pristane and phytane were also present in the test mixture.

Oil degradation in the microcosms

n-Alkanes

For clarity, we organized the compounds from C_{14} to C_{33} into two groups. The first group included those from C_{14} to C_{18}. The second group consisted of the hydrocarbons from C_{19} to C_{33}. This division was made

Table 3 Percentage recoveries from the experimental setups

% recovery	June	July	August	September	October
Aliphatic hydrocarbons	52.1	83.6	90.5	58.5	98.8
Polycyclic aromatic hydrocarbons	47.2	51.7	60.1	58	62

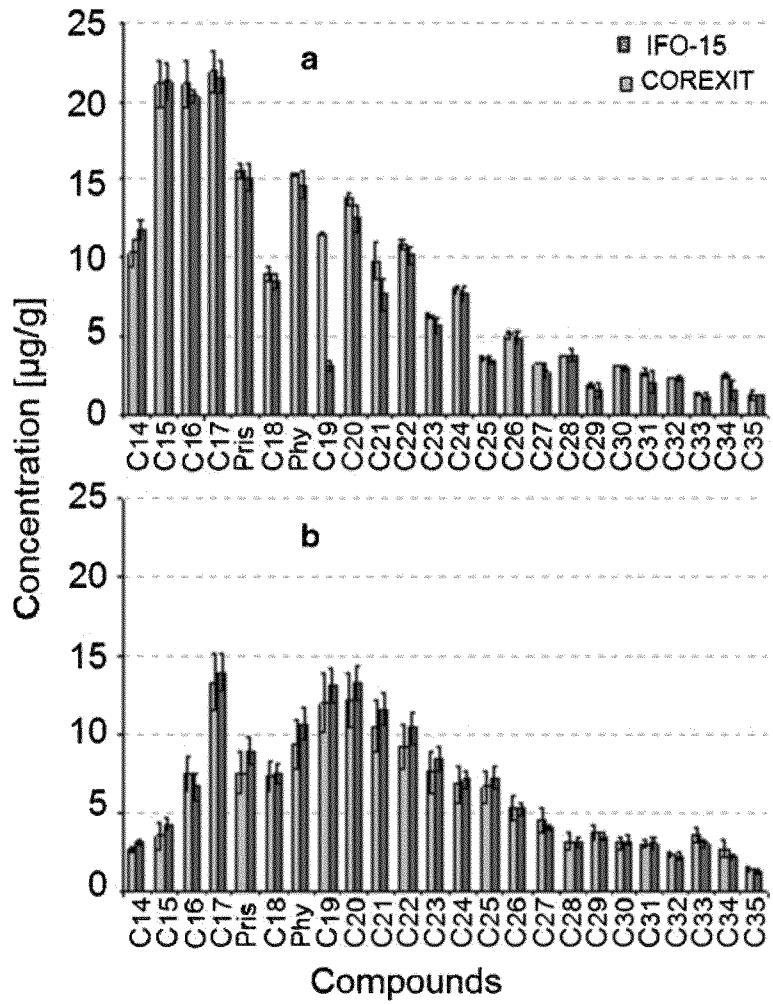

Fig. 4 Concentrations of n-alkanes during the first sampling, 15 days after the initiation of the experiment (a), and concentrations measured at the end of the experiment, which was 120 days from the beginning (b)

because the hydrocarbons from each group behaved similarly during the experiment.

Figure 4 shows the concentrations and distributions for n-alkanes at the beginning and at the end of the

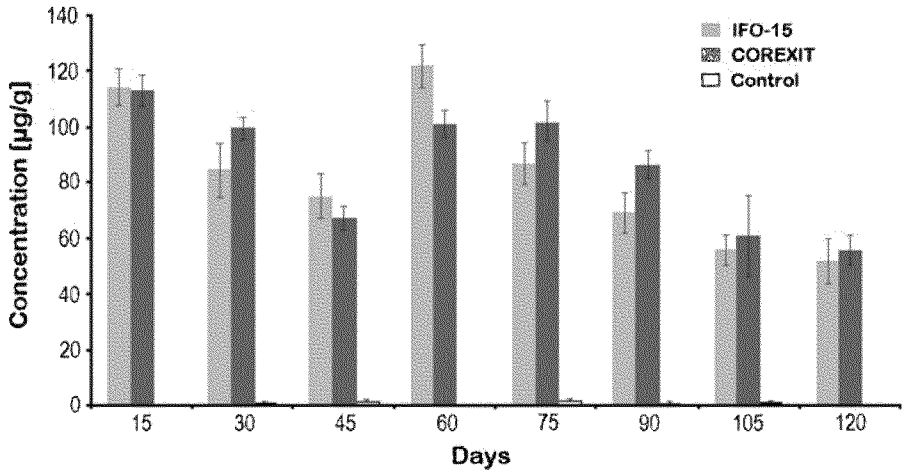

Fig. 5 Sum of n-alkanes for the first group (low molecular weight). It also includes pristane and phytane for the three treatments

experiment. The largest changes appear to occur in the shortest or lowest molecular weight molecules. This is consistent with the expectations that low molecular weight un-branched compounds degrade easier than larger compounds (Scott and Nelson 2004; Leahy and Colwell 1990).

The concentration for the first group of n-alkanes showed fluctuations along the duration of the experiment. As such, we are presenting a curve that describes the changes in a chronological order.

In Fig. 5, we show the biweekly behavior of hydrocarbons (medium-sized MW), showing a decay that began immediately during the first sampling. However, there is also an apparent increase after the third sampling (60 days). We hypothesize that the fluctuation may have been related to un-homogeneous distribution of hydrocarbons in the sediment. Once that the "new" sediment concentration was exposed, the degradation continued till the last sampling, There were some fluctuations at

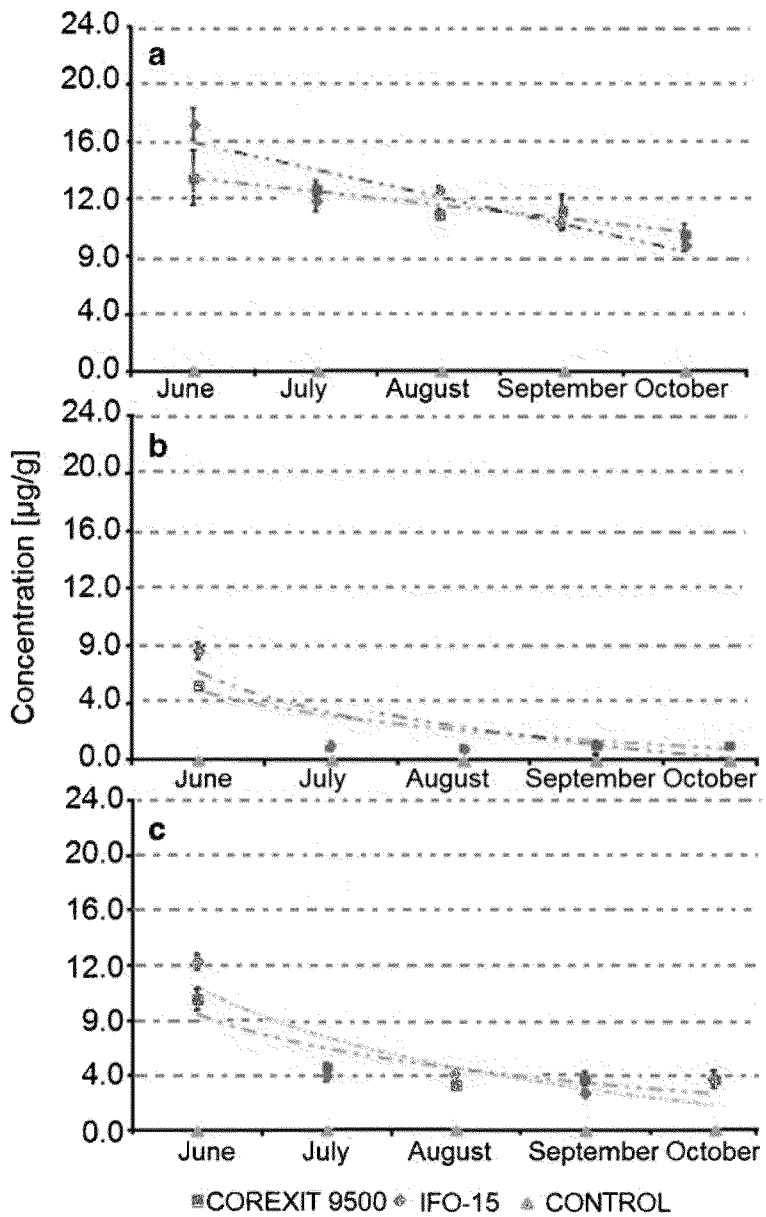

Fig. 6 Change in concentration for Pyrene (a), Benzo[a] anthracene, (b) and Chrysene (c) during the 5 months long experiment. Comparison between treatments, all values are average for three replicates. The changes are small and hardly distinguishable. IFO-15 is indicated in blue and the treatment with Corexit© in red

the last samplings; however, the overall trend suggests a decomposition of the hydrocarbons for both treatments of about 50 % with respect to the initial concentration. In Fig. 5, we also show the distribution of the LMW compounds during the experiment.

Polyaromatic hydrocarbons

Most PAHs showed smaller changes during the time frame of the experiment. At the same time, there were fluctuations in concentration, probably due to uncertainties in measurements. The decay behavior of decomposition was very similar regardless of the presence or absence of the dispersant, as observed in Fig. 6a–c.

Other aromatics showed a non-linear apparent decomposition, as seen in Fig. 6b (benzo[a]anthracene) and Fig. 6c (pyrene).

We observed that the dissolution of compounds already in the sediment was minor, even with the presence of the dispersant. It was also determined that the aliphatic compounds were more susceptible to degradation for the duration of the experiment than the PAH group. However, there were important differences.

As apparent in Fig. 5, within the aliphatic compounds, those of low molecular weight were most easily degraded. On the contrary, for the largest molecular weights (C_{19}–C_{36}), there was no significant change in concentration measured between the initial and final dates.

Significant differences were only noticed (P=0.01, n=7) when the initial and final concentrations are compared for the lightweight normal hydrocarbons (lightweight: from C_9 to phytane). These differences are for the samples containing IFO-15. Similarly, there were also significant differences (P=0.007, n=7) between initial and final concentrations for the same range of hydrocarbons for those samples containing IFO-15 and Corexit 9500©. However, the initial and final concentrations for n-alkanes from C_{19} to C_{36} for the experiments containing IFO-15 did not show significant differences (P=0.14, n=17).

We calculated the percentage degradation for the treatment with IFO-15 that showed a degradation of about 62 %; treatment with Corexit 9500© resulted in 60 % degradation. There were no significant differences in degradation.

The decomposition behavior for n-alkanes appears to be modeled by a first-order decay. This makes it simple to predict the evolution of the decomposition of wasted oil under these conditions and suggests, as expected, that the compounds are generally easy to degrade, at least for the first few n-alkanes in the series. The two most frequent models used to fit the decay are the logarithmic and exponential decay, with the latter being the preferred one in the presence of a bacterial consortium.

With respect to PAHs, the situation is slightly different. The degradation for the treatment with the oil but no surfactant was about 66 %; however, for the treatment that included the dispersant, the degradation was calculated at 47.5 %.

Conclusions

In the contaminated sediment, aliphatic hydrocarbons, particularly those from 14 to 18 carbons, were more susceptible to degradation processes consistent with previous studies (although there were no notable differences between those with dispersant from those without it).

For the aromatic hydrocarbons (PAHs), the removal or degradation appears to be favored when compared to n-alkanes. The presence of the dispersant did not represent an inhibitor for this particular seed used in the experiment and for the conditions of the overall setup, including salinity and temperature.

Based on the results, we could not detect significant differences between treatments. There were differences between compounds because the removal of the short-chained hydrocarbons (C_{14}, C_{15}, and C_{16}) showed larger fluctuations with a variation within treatments of ±5 %.

The dissolution of the hydrocarbons was minor (about 1 %) to the water column with concentrations in the order of a few nanograms per milliliter. This suggests that resuspension due to air injection or sampling disturbance was minimal, even for those treatments containing dispersant.

This degradation experiment's results suggest that the use of Corexit 9500© does not represent a large difference in degradation velocities under conditions similar to those of subtropical environments. We must caution that these are very limited results upon which to base acceptance of the general use of dispersants under all conditions. We also would like to emphasize that within the time frame of the study, the presence of the dispersant did not significantly change the degradation process in the sediment for these two groups of

compounds under the experimental conditions used. There was evidence of a larger percentage of degradation for PAHs than for saturated hydrocarbons. We suggest that this may be partly due to the larger concentrations present for alkyl compounds compared to aromatics. This may have resulted because even partial degradation of large alkylated molecules would result in the production of smaller n-alkane compounds obscuring the degradation of these smaller molecules. On the contrary, for the PAHs that had lower concentrations at the beginning of the experiment, the resulting changes were more noticeable. This behavior has been noticed and reported before. For example, Lepo and Cripe (1999), and references therein, mentioned that the lower molecular weight PAHs were "substantially depleted" under aerobic conditions; in contrast, n-alkanes were not. Our experimental conditions insured an aerobic environment throughout the experiment.

In all experiments, the initial concentration of hydrocarbons appears to be slightly larger for the treatment without dispersant than with dispersant. It appears that the presence of the surfactant makes the hydrocarbons less available for extraction with an organic solvent such as the one used here. The surfactant also appears to make hydrocarbons less available for degradation in the sediment because, in the end, there also appears to be slightly less hydrocarbon degraded in the presence of the surfactant.

Given that the use of dispersants for emergency contingency plans such as Corexit 9500© may be done as close as 5 km from the coast (deeper than 10 m), it is highly probable that it will reach the sediment mixed with oil (NRC, 2005). One of the questions addressed by this work is about the fate of the dispersant and oil mixture in contact with sediments compared to that of naturally dispersed oil. We found no evidence of advantages in the use of the dispersant under conditions similar to subtropical environments; as such, we do not recommend its use where particle interaction is very high. We must however understand that still, there may be aesthetical advantages in removing oil spots from the water column.

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To: Mandsager, Kathy[kathy.mandsager@unh.edu]; Conmy, Robyn[Conmy.Robyn@epa.gov]; 'fingasmerv@shaw.ca'[fingasmerv@shaw.ca]; 'tchazen@utk.edu'[tchazen@utk.edu]; 'Robert Jones - NOAA Federal'[robert.jones@noaa.gov]; 'Samantha Joye'[mandyjoye@gmail.com]; 'ken.lee@csiro.au'[ken.lee@csiro.au]; 'mbleigh@alaska.edu'[mbleigh@alaska.edu]; 'karl.linden@colorado.edu'[karl.linden@colorado.edu]; 'kmmcfarlin@alaska.edu'[kmmcfarlin@alaska.edu]; 'thomas.s.coolbaugh@exxonmobil.com'[thomas.s.coolbaugh@exxonmobil.com]
Cc: nancy.kinner@unh.edu[nancy.kinner@unh.edu]; Kinner, Peter[Peter.Kinner@unh.edu]; Ian P Gaudreau[ipu3@wildcats.unh.edu]
From: Lindsey R Howard
Sent: Wed 3/11/2015 6:25:01 PM
Subject: Re: FW: Dispersant Science in Arctic Waters - Degradation and Fate
Prince Butler 2014.pdf
Kleindienst, Paul Joye 2015.pdf

Attached is Mandy Joye's paper and Prince and Butler 2014.

Thanks,

Lindsey Howard

From: Mandsager, Kathy <kathy.mandsager@unh.edu>
Sent: Tuesday, March 10, 2015 4:57 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'thomas.s.coolbaugh@exxonmobil.com'
Cc: Kinner, Nancy; Kinner, Peter; Ian P Gaudreau; Lindsey R Howard
Subject: FW: FW: Dispersant Science in Arctic Waters - Degradation and Fate

This is a reminder of our WebEx meeting scheduled for tomorrow, beginning at 130pm ET. See the login instructions below. We understand that all of you are not able to participate, but we must keep plodding forward ☺

Thank you!

From: Mandsager, Kathy
Sent: Friday, February 27, 2015 3:45 PM
To: 'Conmy, Robyn'; 'fingasmerv@shaw.ca'; 'tchazen@utk.edu'; 'Robert Jones - NOAA Federal'; 'Samantha Joye'; 'ken.lee@csiro.au'; 'mbleigh@alaska.edu'; 'karl.linden@colorado.edu'; 'kmmcfarlin@alaska.edu'; 'thomas.s.coolbaugh@exxonmobil.com'
Cc: Kinner, Peter; Kinner, Nancy; Mandsager, Kathy
Subject: Dispersant Science in Arctic Waters - Degradation and Fate
Importance: High

Dear Degradation & Fate group members:

Our next meeting to discuss the outstanding items on this document, particularly with information from the older published papers (LUMCON) that address biodegradation, will be held **Wednesday, March 11 beginning at 1:30 pm ET**. Please mark your calendar and plan to participate.

Attached is the biodegradation spreadsheet for this discussion.

This meeting will be via WebEx and the instructions are noted below.

Degradation & Fate Group
Wednesday, March 11, 2015
1:30 pm | Eastern Daylight Time (New York, GMT-04:00) | 3 hrs

Join WebEx meeting

Meeting number: 312 666 165

Join by phone
1-855-244-8681 Call-in toll-free number (US/Canada)
1-650-479-3207 Call-in toll number (US/Canada)
Access code: 312 666 165
Global call-in numbers | Toll-free calling restrictions

Can't join the meeting? Contact support.

Kathy Mandsager

Program Coordinator

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234 Gregg Hall, Colovos Rd

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603.862.1545

Assessing the effects of chemical dispersants on microbial community composition and activity

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Preface

Dispersants are globally and routinely applied as an emergency response to oil spills in marine ecosystems, with the goal of chemically enhancing the dissolution of oil into water, which is assumed to stimulate microbially-mediated oil biodegradation. However, little is known about how dispersants affect microbial community composition or biodegradation activities. The published findings are controversial, likely due to variations in laboratory methods, the selected model organisms, and, potentially, the chemistry of dispersant-oil mixtures. Here, we argue that an in-depth assessment of the impacts of dispersants on microorganisms is needed to provide the

23 robust knowledge necessary for appropriate planning and ex ecuting future dispersant use during
24 oil spill response.
25
26 **Introduction**
27 Microbially-mediated oil degradation is a key environmental remediation activity that is carried
28 out by a wide diversity of microorganisms across assorted marine environments under both
29 aerobic and anaerobic conditions [1] (Box 1). In crude oils, ~33% of the total hydrocarbons are
30 alkanes, with cycloalkanes (e.g. cyclopentane and cyclohexane) and their derivatives (e.g.
31 methylcyclohexane). Aromatic hydroc arbons such as benzene, naphthalene and phenanthrene
32 and their alkylated derivatives, are on average 35% of total hydrocarbons [2]. Alkenes (such as *n*-
33 hexene, *n*-heptene and *n*-octene[3]) are relatively unstable because of their unsaturated chains and
34 are therefore less abundant. Cru de oils also contain a substantial but variable fraction of non -
35 hydrocarbon resins and asphaltenes (e.g. high, >500 dalton, molecular weight polycyclic organic
36 molecules that contain N, S, and O atoms) that can average 14% by weight[2].
37 Crude oil enters marine environments at natural hydrocarbon seeps [4] at a global estimated
38 rate of 700 million liters of oil per year [5]. In most natural seeps, microbial communities are
39 exposed to slow diffusive hydrocarbon fluxes, although some high flux (advective) seeps result
40 in higher exposure rates to gas and oil [6]. Indigenous microorganisms are likely physiologically
41 adapted to natural hydrocarbon seepage[7] and routinely utilize gas and oil -derived hydrocarbons
42 as carbon and energy sources. At natural seeps, gases consist of mainly aliphatic hydrocarbons,
43 while up to 86% of the hydrocarbons present in crude oils are saturated aliph atic and aromatic
44 hydrocarbons[2]. A second source of oil to marine environments is anthropogenic input, which

45 occurs mainly through tank vessel or pipeline spills, estimated in 2003 to be approximately 117
46 and 14 million liters per year, respectively[5].

47 To initiate the breakdown of certain hydrocarbon classes (e.g. PAHs) , microorganisms
48 produce extracellular enzymes[8]. Another feature of various hydrocarbon degraders is the
49 production of biological surfactants (e.g. glycolipids, lipopeptides, lipoproteins, or
50 heteropolysaccharides[9]) which facilitate oil degradation. Biosurfactants act as natural dispersants
51 that emulsify oil in water, making the oil potentially available for biodegradation. The biological
52 production of surfactants in marine ecosystems can also lead to the formation of microbial
53 aggregates, so called oil-containing marine snow[10]. Furthermore, hydrocarbon degrading-
54 microorganisms are part of a complex microbial network that is influenced by biological
55 (grazing, viral lysis) and chemical (nutrient availability) factors . These environmental factors
56 may actuate, limit or even inhibit oil biodegradation[11].

57 As an emergency response to environmental oil spills, chemical dispersants are routinely
58 applied to the contaminated environment [12] to: i) facili tate dissolution of oil into the water, ii)
59 stimulate biodegradation by increasing the surface area of oil, iii) reduce the amount of oil
60 accumulated on the surface, and iv) reduce hydrocarbon delivery to the shoreline ecosystems.
61 However, the impacts of d ispersants on microorganisms, their activity , and ecology remain
62 largely unexplored.

63 Previous laboratory and field studies assessing the impacts of dispersants on microbial
64 communities have generated inconsistent results, concluding that dispersants inhibit[13], have little
65 to no impact[14,15], or stimulate[16] biodegradation. Furthermore, it was shown that microorganisms
66 utilize dispersants as growth substrates[17,18]. Research studies investigating the impact of
67 dispersants on microbial communities or cultured representatives are limited and there is no

68 definite evidence of overall positive or negative effects of dispersants on microbial oil
69 biodegradation activity. This Opinion summarizes t he current knowledge of the impacts of
70 dispersants on microorganisms and identif ies critical knowledge gaps that should guide future
71 research efforts.
72
73 **Dispersant applications**
74 There are 23 U.S. Environmental Protection Agency (EPA)-approved commercially di spersants
75 available for use in oil spill response efforts, including COREXIT®EC9500A,
76 COREXIT®EC9500B, COREXIT®EC9527A, Finasol®OSR52, and JD-2000™. Dispersants are a
77 mixture of solvents and surfactants. The dispersant COREXIT contains 50% hydrocarbons, 40 %
78 glycols, and 10% dioctylsulfosuccinate (DOSS)[19,20]. COREXIT EC9500A and COREXIT
79 EC9527A contain varying amounts of hazardous compounds, including organic sulfonic acid salt
80 (10-30% w/w) and propylene glycol (15% w/w), while COREXIT® EC9500A also includes
81 hydrotreated light petroleum distillates (10 -30% w/w) and COREXIT® EC9527A contains 2 -
82 butoxyethanol (30-60% w/w)[19,20].
83 When dispersants are applied to oil in aqueous media, their surface–active agents
84 (surfactants) stabilize the oil droplets: at the water -oil interface, the hydrophobic portion of the
85 surfactant molecule orients toward s the oil phase while the hydrophilic part orients towards the
86 water phase (FIG. 1 a, b). The oil becomes emulsified and large clumps are converted into
87 droplets ranging in size from micron s to millimeters (FIG. 1c), reducing the interfacial tension
88 and increasing the surface area. The fate of dispersants and oil is dependent on a variety of
89 factors (FIG. 2), including the dispersion efficiency of oil droplets into the water, the physical
90 distribution of the dispersed oil, interaction of dispersed components with particulate organic

matter, biodegradation, dissolution, evaporation, emulsification, and oil -stranding on shorelines, which can shift the primary mode of biodegradation from aerobic to anaerobic. The effectiveness of dispersants, which is often defined as the ratio of dissolved oil to oil accumulated on the sea surface, depends on various environmental factors, such as temperature, salinity, and light (the latter is only relevant in surface waters)[21] but also strongly depends on the composition of oil[22,23].

The quantity of dispersants applied during marine oil spills is often lacking in reports[12], yet the recommended dose for Corexit is 1:10 (dispersant/oil, v/v[24]). Whether this goal is achieved in the field remains questionable because quantification of the dispersant to oil ratio is challenging under environmental conditions (e.g. when dispersants are administered at a gushing subsurface wellhead using a pressurized hose or when dispersants are applied from airplanes). Additionally, the number of spill s to which dispersants have been applied is difficult to estimate because the documentation about their application is not always publicly available. Nevertheless, some dispersant applications are well -documented: from 1968 to 2007, there were more than 213 described instances of dispersant applications [12]. Intense scientific debate over dispersant usage began in in 1967, when the T/V Torrey Canyon, a 300 m long supertanker, ran aground off the coast of England . The tanker spilled approximately 120 million liters of crude oil, causing an environmental disaster in the Atlantic near the Isles of Scilly. The dispersant BP1002 was applied to contaminated areas using vessels and helicopters and the dispersant-oil mixture was lethal to numerous sea birds and mari ne organisms. The chemical composition, rather than the amount, of dispersants was concluded to trigger the observed animal mortality and this event underscored the need for less toxic dispersants. The impact of the dispersants on marine microorganisms was not assessed.

Subsequently, the chemical composition of dispersants was altered to reduce negative effects on biological organisms although chemical dispersants were still widely used after oil spills. For instance, in March 1979, the well Ixtoc I exploded, leading to a massive discharge of gas and oil into the Gulf of Mexico [25]. In response, 4-10 million liters of COREXIT® EC9527A were applied. While the ecological impacts of this well blowout are well described[25], the observed effects were argued to result from oil exposure and no assessment of dispersant effects was done. Subsequent oil spills were routinely treated with up to 110,000 liters of COREXIT® EC9527A or COREXIT® EC9500A. These spills included vessels that were involved in accidents (e.g. T/V Exxon Valdez, M/V Sea Empress, T/V Evoikos, T/V Red Seagull and M/V Blue Master) and pipeline accidents (e.g. the Vastar platform, the Jesse Pipeline, the High Island Pipeline, the BP-Chevron Pipeline and the Poseidon Pipeline). The recent explosion and sinking of the Deepwater Horizon (DWH ; Box 2) drilling platform, in 2010, resulted in an unprecedented application of dispersants to the surface and, for the first time, deep waters of the Gulf of Mexico.

The efficacy of dispersants has been evaluated in some field studies in both offshore (North Sea off the Eastern Coast of the UK,)[26] and nearshore (Long Cove, Searsport, Maine)[27] habitats. The results of these studies showed that dispersants were effective at removing oil from the surface (i.e. increasing the dissolved oil fraction), but little to no information of the microbial responses and dynamics following dispersant application was obtained , leaving the unanswered question of whether dispersants actually stimulated microbial oil biodegradation. The DWH incident is the first marine oil spill for which comprehensive data are available, regarding the microbial community response to oil injection and dispersant application (e.g. [13,24-32]; see detailed discussion below).

136

137 **Stimulation or inhibition of oil degradation?**

138 Microbial hydrocarbon degradation rates commonly depend on the chemical structure of the
139 hydrocarbon compound (e.g. alkanes, cycloalkanes, PAH), the pathway of hydrocarbon
140 degradation (e.g. aerobic or anaerobic), the hydrocarbon concentration, and the metabolic
141 potential of the microbial population. The implicit assumptions are that dispersants stimulate
142 natural aerobic oil biodegradation by converting large oil m olecules into micron -sized droplets
143 that are more readily degraded , and that natural levels of essential nutrients and oxygen are
144 sufficient to support microbial degradation of th is dispersed oil[28]. However, blue water pelagic
145 systems are nutrient limited[29] and large inputs of dissolved organic carbon in the form of
146 dispersed oil to the surface ocean could exacerbate nutrient limitation and restrict oil
147 biodegradation, independent of the oil droplet size[30]. In sediments, the addition of
148 COREXIT® EC9500A had little effect on the degradation of aromatic or aliphatic hydrocarbons ;
149 once the oil had penetrated the sediment, no significant differences were found between
150 dispersed oil vs. oil treatments[15]. Dispersant stimulation of hydrocarbon degradation is prop osed
151 to stem mainly from increasing concentrations of bioavailable hydrocarbons. This assumes,
152 however, that the only factor limiting biodegradation is oil availability and that dispersants exert
153 no negative consequences on microorganisms and microbial activity. In fact, whether oil
154 biodegradation rates are enhanced by dispersants is largely unknown.

155 Previous studies described the effects of several commercially available dispersants but the
156 results differed among studies . For instance, COREXIT ® EC9527A had varying impacts on
157 alkane degradation and no clear effect on aromatic degradation in marine oil-degrading
158 populations grown on Prudhoe Bay oil[14]. The microbial response was dependent on nitrogen and

159 phosphate levels and under nutrient -limited conditions, Corexit's inhibitor y effects on alkane
160 degradation were more pronounced. Another study showed that a specific component of
161 dispersants, Span 80, stimulated alkane degradation, while other components, namely DOSS,
162 reduced rates of alkane degradation [13]. The degradation rates of ^{14}C-acetate and ^{14}C-hexadecane
163 by *Acinetobacter calcoaceticus* and *Rhodococcus* sp. strain 094 were evaluated under the
164 influence of different dispersants and crude oil , indicating that surfactants inhibited acetate
165 uptake and oxidation systems in these species[13]. The authors concluded that a mixture of
166 surfactants may independently affect various sites in the cell or, alternatively, surfactants may
167 synergistically influence each other's interactions with cells. Overall, from these studies one
168 could conclude that the presence of specific dispersant compounds (e.g. DOSS, Span 20, Span
169 80, Tween 80, and Tween 85) or COREXIT®9527 likely inhibited oil biodegradation rates.
170 Interestingly, the effect of dispersants on biodegradation of a specific hydrocarbon is
171 probably not predictable by the hydrocarbon class , and dispersants themselves may be oxidized
172 preferentially. A marine microbial consortium mineralized COR EXIT®EC9500A most rapidly,
173 followed by fresh oil, and only then weathered oil and dispersed oil[17], showing that
174 COREXIT®EC9500A is selectively mineraliz ed and that dispersed oil does not always degrade
175 more rapidly than fresh oil. In the same study, month -long incubations (35 days) favored the
176 mineralization of particular oil sub-components (e.g. 2-methyl-naphthalene, dodecane,
177 phenanthrene, hexadecane, and pyrene). After the addition of dispersant, mineralization of
178 hexadecane and phenanthrene was inhibited while mineralization of dodecane and 2 -methyl-
179 naphthalene was unaffected[17]. Taken together, these results led the authors to argue that
180 environmental use of Corexit®EC9500A could either increase or decrease the toxicity of residual
181 oil through selective microbial mineralization of specific hydrocarbons.

182 Experiments conducted with seawater microcosms amended with oil , versus oil-dispersant
183 mixtures or dispersant alone, showed no substantial differences in microbial community
184 composition based on denaturing gradient gel electrophoresis of 16S rRNA gene amplicons[31].
185 An increase in cell density in the first 24 h was observed in all treatments and cell numbers
186 remained high in oil -dispersant and dispersant amended treatments. As noted previously by
187 others, these authors concluded that dispersants were likely used as a substrate or nutrient source
188 by certain microbial groups while at the same time directly or indirectly inhibiting growth of
189 other microbial groups. These results suggest that the effects of chemical dispersants on
190 microbial communities are possibly more negative than the effects of oil alone.
191 In another study, bioreactors containing seawater and BRENT crude oil (a trading
192 classification of sweet light crude oil) demonstrated that oil biodegradation was suppressed by
193 COREXIT®EC9500A, while a biosurfactant, a rhamnolipid, stimulated oil degradation[32]. On the
194 other hand, biodegradation of a different cr ude oil (light crude oil; Shell Refining Company) in
195 seawater was slightly enhanced when oil was dispersed with COREXIT ®EC9500A compared to
196 undispersed oil[33].
197 Clearly, there is no definite answer to whether dispersants stimulate or inhibit microbial oil
198 biodegradation. The ambiguous findings may derive from a lack of standardized laboratory
199 protocols. Other explanations include the use of different types of oil or dispersants, the
200 concentrations of oil and dispersants used in the experiments , the availability of nutrients, and
201 the choice of water samples used in the experiments, which influence s the metabolic potential of
202 the microbial communities. An alternative explanation is that dispersants are simply ineffective
203 at stimulating oil biodegradation in marine waters. Since dispersants can be a preferred substrate
204 for microbial growth, enhanced microbial activities such as increasing carbon mineralization

205 (e.g. CO_2 accumulation) in oil -dispersant experiments could result from mineralization of the
206 dispersant[34] and this could be misinterpreted as stimulation of oil biodegradation. Based on the
207 current knowledge, the impacts of dispersants on microbial community composition and
208 microbial oil-biodegradation activity and efficiency are unpredictable.
209
210 **Physiological and molecular effects of dispersants**
211 The US National Research Council stated in a 2005 report that acute and sublethal toxicity from
212 exposure to dispersed oil are not sufficiently understood[35]. In 2014, little progress has been made
213 and the potential toxicological mechanisms remain largely unexplained.
214 Several studies have described the toxic effects of dispersants on viruses[36] and
215 eukaryotes[37]. However, surprisingly little data are available to describe toxic effects of
216 dispersants on microorganisms. Toxicity assays are often carried out using *Vibrio fischeri*, a
217 bioluminescent marine bacterium found predominantly in symbiotic associations. *V. fischeri* is
218 considered a model microorganism for toxicity tests, and assays with this organisms have been
219 carried out with wastewater [38-41], sediments[42-44] and soil[45]; however, this model organism is not
220 abundant in environments impacted by oil spills such as the DWH, questioning the relevance of
221 this model system. Nonetheless, toxicity tests with *V. fischeri* treated with different dispersant
222 and oil combinations showed that dispersed oil was less or equally toxic compared to oil alone[37].
223 The observed toxicity was proposed to derive from soluble, volatile hydrocarbon components
224 rather than colloidal petroleum hydrocarbons. The authors concluded that dispersed-oil toxicity
225 would be significantly less in systems where dilution produces rapidly declining exposures, e.g.
226 below the surface where dispersed oil is diluted, compared to relatively long, continuous
227 exposures, e.g. in concentrated surface slicks. A very recent study investigated the effects of

228 dispersants on the ammonia-oxidizing bacterium, *Nitrosomonas europaea*[46]. While dispersants
229 alone were not toxic, dispersants increased the toxicity of oil supplied in form of chemically
230 enhanced water-accommodated oil fraction s (CEWAFs) significantly. Over -expression of the
231 NE1545 gene, a marker for aromatic hydrocarbon exposure , in *N. europaea* cells expo sed to
232 WAF and CEWAF suggested that aromatic hydrocarbons are bioavailable to the cells and that
233 PAHs play a major role in the observed inhibition and toxicity.

234 In general, inhibitory effects of dispersants on microorganisms could occur from the
235 dispersant components and/or from the dispers ant-oil mixture, e.g. elevated concentrations of
236 toxic PAHs. In terms of dispersant -derived components, the surfactant molecular structure and
237 the ionic state of the surfactant as well as the solvent type and the aromatic content of the
238 dispersant likely influence toxicity effects. While the chemical composition of dispersants has
239 evolved over time and the dispersant formulations used today are far less toxic than earlier
240 formulations[47], there are indications that even present -day dispersants generate inhibitory effects
241 that are potential ly toxic in nature. In a comparative study using different dispersants, the most
242 toxic component of the dispersant was the surfactant, which was hypothesized to interact with
243 biological membranes[48]. In terms of the oil alone, the most toxic components after aromatics are
244 the saturated hydrocarbons, followed by glycol ethers and finally the alcohols.

245 The exact mechanism s of toxicity effects at the cell - or molecular-level are to the best of
246 our knowledge loosely defined and, thus, require further research. However, knowledge of the
247 chemical composition and chemistry of dispersants [19,20] allows prediction of potential toxicity
248 effects resulting from physical contact with dispersant compounds, namely uptake of dispersants
249 by the cell and a ccumulation of dispersants at the outer membrane or within the cell. Dispersants
250 may physically alter the function of membrane lipids or of specific membrane -bound proteins,

which would disrupt cell membranes and interrupt ATP synthesis, leading to energy limitation. Alternatively, dispersants may interfere with cell membrane surface receptors. Toxicity could also result from chemical reaction with cellular components or irreversible blockage of enzyme active sites. Lastly, dispersants have the potential to exert mutagenic effects. These proposed impacts could arise in hydrocarbon degraders and non-hydrocarbon degraders similarly.

Dispersant impacts following *Deepwater Horizon* discharge

The unprecedented application of dispersants during the DWH discharge stimulated research on potential microbial impacts, with studies carried out in the laboratory and directly in the field. Similar to the studies conducted prior to the DWH discharge, contradictory findings were reported. The effects of COREXIT®EC9500A on microorganisms isolated from DWH oil-contaminated beach sands from Elmers Island included reduced bacterial production and reduced viability of *Acinetobacter venetianus* and *Marinobacter hydrocarbonoclasticus* in the presence of the dispersants compared to dispersant-free controls[49]. Dispersant concentrations were comparable to those observed during the spill (0.00 - 100 $\mu g\ L^{-1}$)[49] and the addition of dispersant resulted in a significant reduction of the live/dead cell ratio for all tested isolates, with the exception of *Vibrio sp*., which appeared to tolerate dispersants. The production and viability of *Marinobacter sp.*, a natural hydrocarbon degrader, was reduced by nearly 100%, suggesting a toxicity effect. The authors concluded that dispersants are potentially highly toxic to the microbial communities directly involved in natural hydrocarbon bioremediation[49].

Deep-sea bacteria enriched by oil and dispersants in laboratory experiments [16] degraded 25% of dissolved Macondo oil without addition of COREXIT® EC9500A and 60% with addition of COREXIT over 20 days; the hydrocarbon components of COREXIT were also degraded.

274 *Colwellia* and *Oceanospirillum* were enriched and subsequently, *Colwellia* strain RC25 was
275 isolated on Macond o oil, indicating its capability to degrade hydrocarbons under laboratory
276 conditions[16]. Another study concluded that dispersants were not toxic to indig enous microbial
277 communities at concentrations comparable to those in the discharge zone [18] based on laboratory
278 results showing that the bacterial isolates *Colwellia* strain RC25 and *Alcanivorax* strain 31
279 degraded various components of COREXIT®EC9500A[18]. *Cowellia sp.* and *Alcanivorax sp.*
280 degraded DOSS and dipropylene glycol *n*-butyl ether, respectively[18]. Given that *Colwellia*
281 became abundant in the DWH deep -water plume over time, it is possible that these microbes
282 biodegraded the dispersants and/or dispersed oil.

283 In another mesocosm experiment using offshore waters, the influence on oil, dispersant
284 and dispersed oil on carbon cycling between viruses, microorganisms, phytoplankton and
285 microzooplankton was examined. In dispersant treatments, an increase in total bacterial biomass
286 was observed but no ciliate response was detected over time , indicating reduced or blocked
287 carbon flow to higher trophic levels, which could have compromised the food web[50]. Laboratory
288 column experiments condu cted with permeable beach sands from Santa Rosa Island, Florida
289 revealed that COREXIT ® EC9500A addition increased the mobility of Macondo oil -derived
290 PAHs by up to two orders of magnitude [51] leading to deeper penetration of PAH into anoxic
291 sediments. Since anaerobic biodegradation rates are often lower than aerobic biodegradation
292 rates, this could potentially increase the environmental lifetime of harmful PAHs.

293 During and after the DWH discharge, the general toxicity and mutagenicity of Macondo oil
294 and COREXIT ® EC9500A were explored using three microbial toxicity assays: the Microtox
295 assay[52] utilizing *V. fischeri*, the QwikLite[53] light production assay using the phytoplankton
296 surrogate *Pyrocystis lunula*, and a mutagenesis assay based on lysogens of *E. coli*. The Microtox

297 assay demonstrated potentially more lethal impacts on bacteria by both oil and oil -dispersant
298 mixtures while the QwikLite indicated that dispersant and dispersant -oil mixtures were more
299 toxic for phytoplankton. In addition, the mutagenesis assay results showed the most sensitivity to
300 pure oil additions [54]. These results [54] document that dispersants, oil, and oil -dispersant mixtures
301 have potentially complex and unpredictable, negative effects on mari ne microbial communities.
302 The microorganisms in contact with these mixtures might experience DNA damage that could
303 lead to mutation and heritable alterations of the microbial community. While mutagenic seawater
304 was detected in August of 2010 in the vicinity of the DWH oil spill well site, no mutagenic
305 effects were observed on the west Florida Shelf[54]. However, in June and November 2011 – more
306 than a year after the Macondo wellhead was capped – mutagenic seawater was observed on the
307 West Florida Shelf, suggesting that the effects of oil -dispersant mixtures on m arine planktonic
308 communities lingered for up to 1.5 years[54]. Since the oil was principally bound with the
309 COREXIT at this time, it appears likely that the mutagenicity resulted from ex posure to oil -
310 dispersant mixtures[54].
311
312 **Future research needs**
313 Of the 23 dispersants currently approved by the EPA for use in oil spill response[55], to the best of
314 our knowledge none has been tested thoroughly to evaluate the effect on microbial communities
315 under natural conditions. Thus, based on currently available information, the utility of
316 dispersants as a stimulant for microbial oil degradation through chemical oil dispersion in the
317 environment is questionable. Risks and potentially negative effects need to be carefully
318 evaluated in the laboratory as well as in the field. Research should be conducted using
319 standardized protocols and methods, to eliminate any potential methods -associated artifacts. For

instance, a crucial methodological aspect is the preparation of oil and dispersant and their addition to samples. WAFs and CEWAFs are ideal to provide physically or chemically dispersed water-soluble hydrocarbons to aqueous samples[56]. The development and establishment of standardized protocols for the preparation of WAF and CEWAF including quality controls for these fractions (analytical measurements of e.g. total petroleum content) would provide comparable results among laboratories and studies. Protocols for WAF and CEWAF preparation and quality testing were published about 15 years ago[56], in the meantime important improvements were discovered and an updated protocol should be developed collaboratively by the research community.

Directed studies testing dispersants effects after the DWH oil spill hypothesized an increased toxicity for specific microbial taxa (e.g. *Marinobacter*[49,57]) as well as toxicity for certain components of the microbial food web [50]. These data suggested that negative impacts might propagate and influence the pelagic ecosystem. One strategy for toxicity tests is to start with the assessment of model organisms that are characteristic of the ecosystem in question, ideally isolated from these particular environments using WAF, CEWAF and dispersants-only. The selection of model organisms for testing dispersants toxicity effects will significantly influence findings because the sensitivity of individual microbial strains is almost certainly variable. This is likely one of the reasons for contradictory results obtained in prior studies. Therefore, the ultimate goal would be to study toxicity effects of complex microbial communities. In this respect, systematic studies are necessary to address direct and indirect effects and acute as well as chronic exposure regimes. For instance, future studies could assess the impact of WAF, CEWAF and dispersants-only on alterations of the microbial community structure, microbial physiology and activity in different habitats such as surface water, deep

343 water, deep-sea sediments as well as beach and marsh sediments. Impacts could be monitored in
344 time course experiments by the analyses of phylogenetic marker genes, dispersant and
345 hydrocarbon concentrations as well as hydrocarbon oxidation rates. The mineralization of
346 dispersant compounds may in fact be as significant as the mineralization of hydrocarbons [34] and,
347 thus, dispersant degradation should be assessed in parallel. The impact of dispersants could
348 furthermore be evaluated by applying 'omics' -techniques, specifically metatransciptomics and
349 metaproteomics, which may offer the opportunity to discover novel biomarkers related to
350 dispersant research. In addition, toxicity and mutagenicity need to be evaluated using established
351 or further optimized toxicity tests (e.g. the development of methods with more relevant model
352 organism).

353 The response of microorganisms is likely further driven by the type of dispersant, the type
354 of oil, and by environmental conditions. These three factors are likely interactive, making
355 prediction of impacts difficult and underscoring the necessity of meticulous experimental design,
356 statistical analysis and modeling of the obtained data to evaluate the influence of these factors,
357 respectively. Direct comparisons of the impacts of dispersants applied to different types of crude
358 oil are necessary si nce light and heavy oils, fuel oil or diesel respond differently to dispersant
359 applications. Future studies should also address the influence of differing environmental
360 conditions such as nutrient availability, temperature, pressure, and salinity. In addition,
361 experiments need to be conducted under light and dark conditions to elucidate whether dispersed
362 oil shows enhanced photo-toxicity.

363 The persistence of dispersants in the environment is another important future research area ,
364 as these compounds are kno wn to persist for years after application [58]. It is critical to analyze the
365 concentrations of oil and d ispersants in all Gulf of Mexico environments that were impacted by

366 the DWH discharge. Crucial questions include: how much of the dispersant components
367 accumulated and persisted in the environment (e.g. marine and beach[51] sediments) and whether
368 the endemic microbial community is metabolizing it? Further, which microorganisms inhabit the
369 environment under these conditions?

370 Finally, the research and development of potential alternatives to currently available
371 dispersants should be encouraged. A recent study promoted the application of a hydrophobically-
372 modified biopolymer as a promising appro ach to reduce the amount of dispersants after oil
373 spills[59]. While chitosan, which also needs to be carefully tested for cytotoxity, was used in that
374 particular study[59] future research in these and similar areas may discover dispersant alternatives
375 that may be beneficial from both an economic and an environmental point of view.

376 We are optimistic that well -conceived future studies will enable critical evaluation of the
377 impacts of dispersants on endemic microbial communities permitting the community to reassess
378 whether dispersants should be recommended after oil s pills in a variety of ecosystems including
379 terrestrial, near shore and deep-sea habitats.

380

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387

388

Boxes

Box 1. Microbial hydrocarbon degradation

Globally relevant aerobic aliphatic and aromatic microbial hydrocarbon degraders affiliate mostly with the *Gammaproteobacteria* (FIG. Box 1 , e.g. *Alcanivorax* spp.[60], *Cycloclasticus* spp.[61], *Oleiphilus* spp.[62], *Oleispira* spp.[63], *Thalassolitus* spp.[64], and *Planomicrobium* spp.[65]). In marine sediments, where anaerobic conditions dominate within a few mm or cm from the sediment surface, sulfate -reducing bacteria affiliating primarily with the *Deltaproteobacteria* (e.g. *Desulfosarcina/Desulfococcus* spp.)[66-68] are key players in hydrocarbon biodegradation. The rate of hydrocarbon biodegradation depends mainly on the physiological capabi lities of the microorganisms and the hydrocarbon class; aerobic processes are generally more rapid than anaerobic processes, while low molecular weight hydrocarbons are degraded at higher rates than high molecular weight hydrocarbons.

Hydrocarbon oxidizer s possess diverse metabolic capabilities including a variety of key enzymes (e.g. oxygenases and glycyl radical enzymes[69]), which enable them to oxidize hydrocarbons either completely to carbon dioxide or incompletely to oxidized intermediate metabolites. The range of hydrocarbons used aerobically and anaerobically by microorganisms is often restricted to a narrow range of ch ain lengths or even only a single compound, making most hydrocarbon degraders specialists rather than generalists. For instance, aerobic aromatic hydrocarbon degraders such as *Cycloclasticus* use naphthalene, phenanthrene, anthracene, and toluene as carbon and energy source[61]. Anaerobic hydrocarbon-oxidizing sulfate-reducing bacteria may degrade C_{3}-C_{20} n-alkanes, C_{7}-C_{23} n-alkenes or aromatic hydrocarbons such as benzene, toluene or naphthalene (see review by Widdel et al .[70] and references therein). In contrast, the aerobic hydrocarbon degrader *Alcanivorax borkurnensis* oxidizes an exceptionally

broad range of hydrocarbons, including linear alkanes (C_5-C_{16} alkanes), isoprenoids (e.g. phytane), alkylarenes and alkylcycloalkanes. Annotation of *A. borkurnensis*'s proteome provides insight that alkane degradation proceeds via several routes of terminal oxidation, in volving AlkB hydroxylases, a putative flavin -binding monooxygenase, and P450 cytochrome(s) [71], giving this microorganism a potential ecological advantage over specialists.

Box 2 | The *Deepwater Horizon* oil spill

During the 2010 DWH hydrocarbon discharge in the northern Gulf of Mexico, 795 million liters oil were released into the deep ocean [72] and about 7 million liters of chemical dispersants were used as a primary response action[73]. Shortly after the discharge began, a hydrocarbon plume was detected between water depths of 1000 to 1300 m[74,75]. The plume likely formed through the nature of the discharge, e.g. physical and chemical factors related to the high -pressure discharge into cold water[76]. Whether plume formation was exacerbated by dispersant application remains debated. The plume microbial communities were exposed to highly elevated hydrocarbon concentrations[77], including low molecular weight alkane gases, benzene, toluene, ethylbenzene, and xylenes (BTEX) [78] and polycyclic aromatic hydrocarbons (PAH) [75] as well as the anionic surfactant dioctyl sodium sulfosuccinate (DOSS)[79,80], a major component of COREXIT. Distinct bacterial groups became successively enriched in the plume, generating large microbial community shifts over time. Members of the bloom microbiota included *Oceanospirillum*, *Cycloclasticus*, *Colwellia*, *Rhodobacterales*, *Pseudoalteromonas* and methylotrophs[78,81-85], which were hypothesized to oxidize hydrocarbons in the contaminated water column. Very recently, it was discovered that the application of dispersants during the DWH oil spill likely altered the microbial community composition in the plume layers[57,86] and the biodegradation rate of

435 hydrocarbons[57]. Furthermore, as much as one-third of the oil was likely mixed with deep ocean
436 sediments, through mechanisms referred to as the dirty bathtub ring[87] (e.g. plume waters
437 intersecting shelf sediments) and the oil-snow blizzard (marine "oil snow" falling to the
438 seafloor[11]). Marine oil snow likely played a key role in the fate of the surface oil during the event
439 of the oil-snow blizzard and, thus, the formation and sinking characteristics of marine oil snow
440 were investigated in laboratory microcosms[88]. Another substantial portion of the oil that reached
441 the seawater surface was transported to coastal ecosystems and buried in sediments, where it is
442 still detectable[89]. In sediments, successional changes in the microbial community composition
443 were detected due to the oil contamination, in both deep-sea surface[34] and coastal sediments[90],
444 and the expression of functional genes involved in hydrocarbon degradation processes[34,90]
445 confirmed that oil was mineralized in these ecosystems as well.
446
447 **Figure Captions**
448 **Figure 1 | Dispersants and their interaction with oil in seawater.**
449 a | Composition of a surfactant molecule, which features hydrophilic and lipophilic components.
450 b | Interactions of dispersants with oil in seawater; the hydrophilic component of the molecule
451 turns toward the seawater while the lipophilic side of the molecule turns toward the oil phase,
452 thus forming small oil droplets that are stabilized by the dispersant. c | As a consequence of
453 dispersants application oil slicks are broken up and oil droplets are dispersed in the water
454 column.
455
456 **Figure 2 | Processes involved in the fate of dissolved and non-dissolved hydrocarbons in the**
457 **water column based on the recent DWH oil spill.**

458 Macondo oil was released from the wellhead and partly dissolved in the water column leading to
459 a hydrocarbon plume at 1000 - 1300 m depth. Oil slicks on surface waters and at the wellhead
460 were treated with dispersants to prevent large surface slick formation. In seawater surface layers,
461 a substantial amount of oil coagulated with bacterial marine snow and sank down to the sediment
462 surface during an event referred to as the oil-snow blizzard. Oil components were widely
463 transported in the Gulf of Mexico; elevated concentrations of hydrocarbons were measured up to
464 hundreds of km away from the source of the contamination and covered and area of
465 approximately 20,000 km. Pelagic microorganisms degraded dissolved hydrocarbons in the
466 contaminated water column, introducing oil components in to the pelagic food web. Thereby,
467 grazers and viruses directly interact ed with hydrocarbon degraders. Some oil likely got stuck to
468 the cells of phytoplankton and zooplankton and was transferred indirectly (e.g. not through
469 ingestion) to higher trophic levels. Persistent oil components sank to the seafloor and got buried,
470 where benthic microorganisms utilize them as carbon and energy source predominantly under
471 anoxic conditions, which generally decelerate biodegradation rates.
472
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A protocol for assessing the effectiveness of oil spill dispersants in stimulating the biodegradation of oil

Roger C. Prince & Josh D. Butler

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Abstract Dispersants are important tools in oil spill response. Taking advantage of the energy in even small waves, they disperse floating oil slicks into tiny droplets (<70 μm) that entrain in the water column and drift apart so that they do not re-agglomerate to re-form a floating slick. The dramatically increased surface area allows microbial access to much more of the oil, and diffusion and dilution lead to oil concentrations where natural background levels of biologically available oxygen, nitrogen, and phosphorus are sufficient for microbial growth and oil consumption. Dispersants are only used on substantial spills in relatively deep water (usually >10 m), conditions that are impossible to replicate in the laboratory. To date, laboratory experiments aimed at following the biodegradation of dispersed oil usually show only minimal stimulation of the rate of biodegradation, but principally because the oil in these experiments disperses fairly effectively without dispersant. What is needed is a test protocol that allows comparison between an untreated slick that remains on the water surface during the entire biodegradation study and dispersant-treated oil that remains in the water column as small dispersed oil droplets. We show here that when this is accomplished, the rate of biodegradation is dramatically stimulated by an effective dispersant, Corexit 9500®. Further development of this approach might result in a useful tool for comparing the full benefits of different dispersants.

Keywords Oil spill dispersants · Crude oil · Biodegradation · Oil spill response

Responsible editor: Robert Duran

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Introduction

Dispersants are important options in the "tool box" for responding to marine oil spills. Developed after initial attempts to disperse oil with simple industrial cleaners (National Research Council 1989), current products are carefully blended mixtures of usually food-grade surfactants and solvents (National Research Council 2005) that can disperse a range of crude oils and refined products (Lessard and DeMarco 2000) over a wide range of conditions ranging from arctic (Belore et al. 2009) to tropical (Zahed et al. 2011).

Several laboratory protocols have been developed for testing the relative efficacy of different dispersants: the air current test (Mackay et al. 1978), the Warren Springs rolling flask test (Martinelli 1984), the swirling flask test (Clayton et al. 1993), the Exxon dispersant effectiveness test (Becker et al. 1993), and the baffled flask test (Venosa et al. 2002) among others. Comparisons of these tests with field and tank data have been reported by Clark et al. (2005), and the baffled flask test was used to compare eight potential dispersants during the response to the Deepwater Horizon spill (Venosa and Holder 2012).

Useful as they are, these are very small volume tests, and they are not amenable to studying long-term processes (days to weeks) such as biodegradation. In practice, dispersants are only used on significant slicks, and the tests use relatively high concentrations of oil to mimic this. In the field, the dispersed oil droplets (typically <70 μm) diffuse apart, dropping to concentrations below 1 ppm in hours to days (Delvigne 1993; Li et al. 2009; Lee et al. 2013), and they do not re-coalesce. Until now, laboratory tests have used volumes that keep the oil concentration in the many hundreds to many thousands of parts per million, and re-coalescence occurs in hours to days.

The ultimate purpose of oil spill dispersants is to dilute spilled oil so that indigenous microbes in the sea can consume the oil. Hydrocarbons are excellent sources of carbon and energy for those organisms, prokaryotic (Prince et al. 2010)

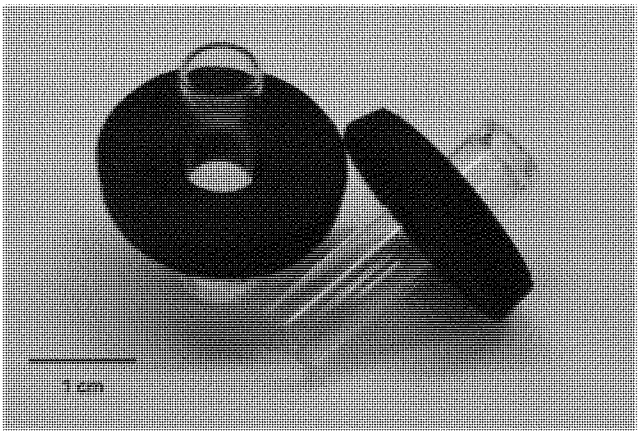

Fig. 1 Glass "booms" to maintain small volumes of oil as a floating slick. The thin glass tubing is held upright by the closed cell ethylene vinyl acetate foam

and eukaryotic (Prince 2010), able to initiate biodegradation, but they lack biologically available nitrogen, phosphorus, and other essential elements for microbial growth. Fortunately, seawater usually provides enough of these nutrients if the hydrocarbons are in the few parts per million concentration or less, and biodegradation of dilute oil has a "half-life" of days to weeks (Hazen et al. 2010; Baelum et al. 2012; Prince et al. 2013).

Quantitatively demonstrating that oil dispersants do indeed stimulate oil biodegradation under controlled conditions has been a major challenge. Current protocols for testing the efficacy of dispersants generate dispersions that are far too concentrated for the available nitrogen, phosphorus, etc. in seawater to allow prompt biodegradation, and experiments are rarely carried out long enough for biodegradation to occur even if the nutrient problem is overcome by adding fertilizer (although see MacNaughton et al. 2003). Conversely, oil at the few parts per million level, where biodegradation is rapid, is dispersed fairly well by laboratory stirring even without adding dispersants, and dispersants have only a minimal stimulatory effect on biodegradation (Prince et al. 2013). Here, we describe experiments where the total oil available in the system is dilute enough that there are potentially enough nutrients in the seawater for rapid biodegradation, but where natural dispersion is slowed by enclosure of the oil in a floating boom unless dispersant is added. These concentrations approximate those found after the successful application of dispersants at sea (Lee et al. 2013), and are below levels where any measurable toxicity occurs, even in 48 (Americamysis bahia) or 96 h (Menidia beryllina) tests (Hemmer et al. 2011). Under these conditions, Corexit 9500, the dispersant used most widely following the Deepwater Horizon spill, dramatically stimulates the rate of oil biodegradation.

Methods

Seawater was collected from the New Jersey shore in August and September, 2012 (summer conditions; salinity=28 ppt, temperature=21 °C). Nitrate and phosphate levels were below detection limits with simple laboratory colorimetric tests, but are likely to have been near 7 and 0.5 μM, respectively (Louanchi and Najjar 2001). The experiments used 4 l of this seawater in 5 l carboys, maintained at 21 °C in a growth chamber with a diurnal light cycle (16 h on, 8 h off). The oil was Alaska North Slope crude (ExxonMobil 2013), weathered by evaporation at laboratory room temperature in a hood

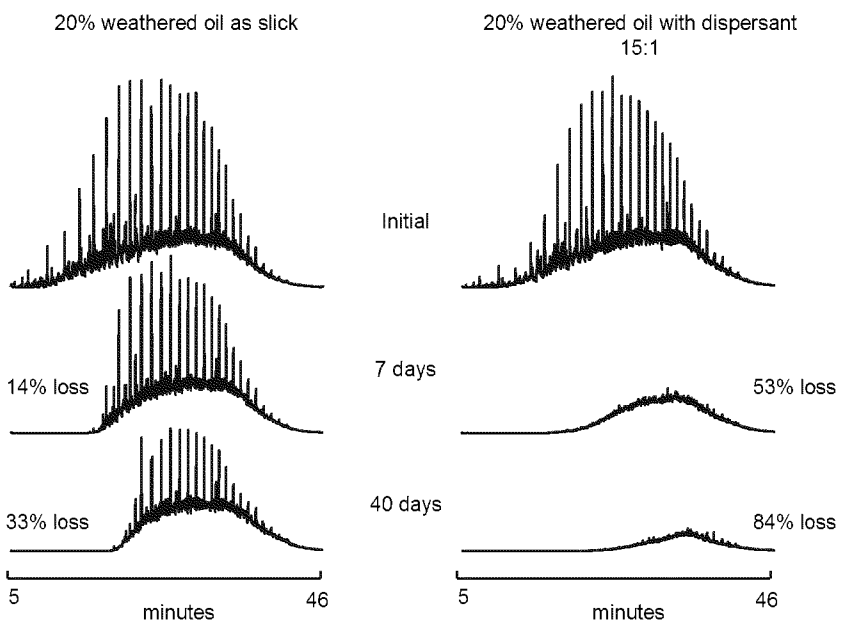

Fig. 2 Total ion chromatograms of the oils (without and with dispersant added at a dispersant to oil ratio of 1:15) extracted after 15 min (initial), 7 and 14 days. The "booms" were removed from the experimental vessels, separated from their flotation collars, and extracted with methylene chloride, as was the water phase. No detectable hydrocarbons were found in the water phase of the oil without dispersant, and none on the glass boom in the samples with dispersant

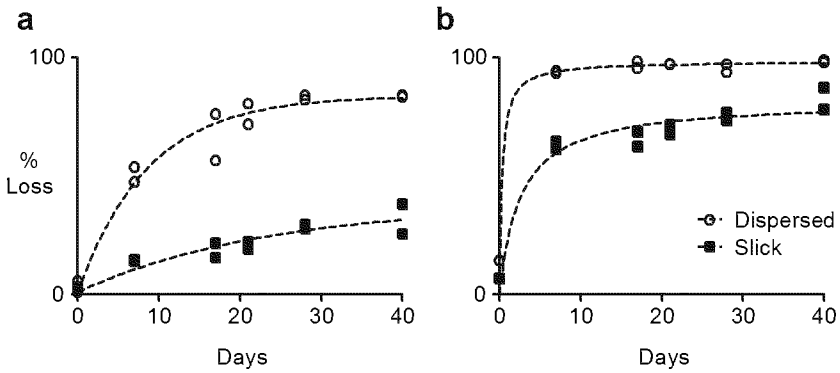

Fig. 3 Loss of a total detectable hydrocarbons and b the sum of the USEPA priority pollutants (Keith and Telliard 1979) from samples with and without dispersant

until it had lost 20 % of its weight; this is a reasonable simulacrum of oil that has weathered between a spill and the application of dispersant from the air (National Research Council 1989, 2005). Oil (10 μl) was added into small floating booms (Fig. 1), some receiving oil alone, others receiving oil that had been premixed with Corexit 9500 at a dispersant to oil ratio of 1:15. The glass booms enclosed a surface area of 28 mm^2, so the 10 μl resulted in a slick with a nominal thickness of 350 μm, although of course there was a meniscus around the edge. The vessels were stirred with a magnetic stirrer to generate a 2-cm vortex, and within minutes, the oil with dispersant had dispersed into the water (to a nominal concentration of 2.5 ppm), while the oil without dispersant remained in the boom. Carboys were incubated up to 40 days, with duplicate vessels with and without dispersant sacrificed at various times throughout the study.

Upon sacrifice, the booms were carefully removed and the carboys extracted three times with methylene chloride. The glass booms were removed from their foam flotation collars and washed twice with methylene chloride. The methylene chloride extracts were collected with a pipette, evaporated carefully to a few milliliters, dried over anhydrous sodium sulfate, and then evaporated to approximately 1 ml, with care to prevent concentration to dryness. In the absence of dispersant, there were no detectable hydrocarbons in the bulk phase, while with dispersant, there was no oil remaining on the glass booms.

Oils were analyzed by gas chromatography coupled with mass spectrometry (Douglas et al. 1992). Hydrocarbon biodegradation was followed with respect to 17α(H),21β(H)-hopane as a conserved internal marker within the oil (Prince et al. 1994).

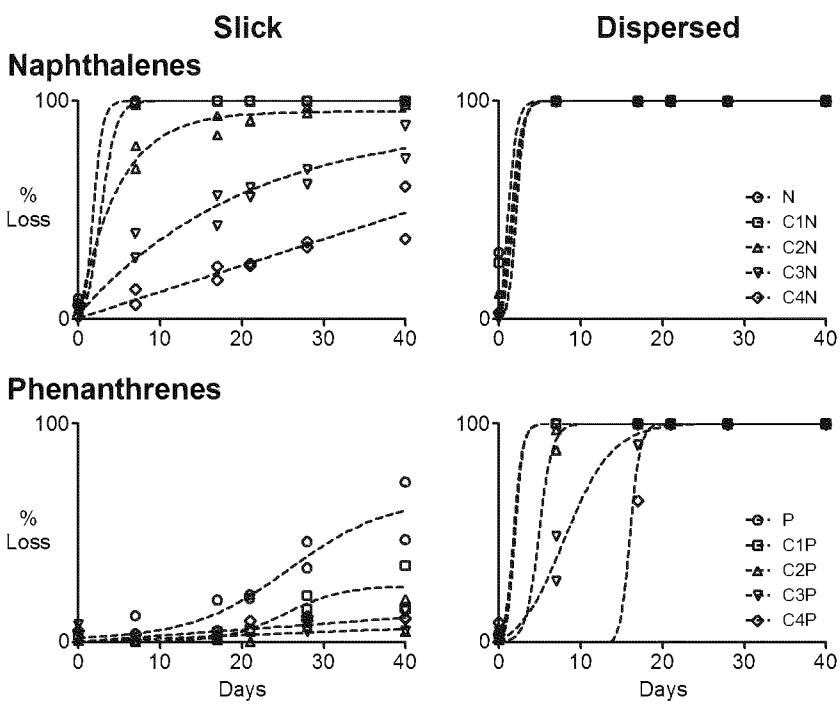

Fig. 4 Loss of naphthalene, phenanthrene, and their alkylated congeners from samples with and without dispersant. N indicates naphthalene, C1N indicates the sum of the methylnaphthalenes, C2N indicates the sum of the dimethyl- and ethyl-naphthalenes, C3N the sum of the trimethyl-, methylethyl-, propyl-, and isopropyl-naphthalenes, etc., while P indicates phenanthrene, etc. We detected no significant preferences for the biodegradation of any isomers over others

Results

Figure 2 shows representative chromatograms of oils extracted at the initiation of the experiment (15 min after the assembly of the experiments) and after 7 and 40 days of incubation. The sharp peaks are the n- and branched alkanes, while the broad features are the "unresolved complex mixture" in which reside the polycyclic aromatic hydrocarbons. It is clear that biodegradation and evaporation are occurring in samples with and without dispersant, but it is obvious that biodegradation is far more extensive in the dispersed oil than in the slick. Within a week, the dispersed oil had lost approximately half of its detectable hydrocarbons, while the slick had lost only 14 %, and by 40 days, the slick had still not degraded as much as the dispersed oil had in 7 days. Meanwhile, primary biodegradation of the hydrocarbons had reached 84 % in the dispersed oil. Figure 3 shows this graphically, with panel a showing loss of total detectable hydrocarbons, and panel b the loss of the polycyclic aromatic hydrocarbons on the USEPA priority pollutant list (Keith and Telliard 1979). Naphthalene is the most abundant of these molecules in this oil, and the loss of this compound can be attributed to both evaporation and biodegradation (Fig. 4), but its alkyl congeners, and the phenanthrenes, are not significantly volatile, and their disappearance (Fig. 4) can be attributed to biodegradation. Clearly, dispersed oil degraded much more rapidly and extensively than undispersed oil.

Discussion

As far as we are aware, this is the first laboratory demonstration of a substantial and dramatic stimulation of the rate of biodegradation of crude oil by the addition of an oil dispersant. At first glance, this seems to contradict a substantial body of work that found only minimal stimulation at best (Van Hamme and Ward 1999; Lindstrom and Braddock 2002; Venosa and Holder 2007; Prince et al. 2013). We believe that this can be entirely attributed to the fact that in those experiments the relatively low concentrations of oil dispersed quite naturally, almost as well as with dispersants, albeit with slightly larger droplet size (Shaw and Reidy 1979). Since biodegradation is likely dependent on the surface area available for microbial colonization, there is only a small increase of accessibility in decreasing droplet size once small droplets have formed. In contrast, going from a floating slick to 70 μm droplets increases surface area by at least 20-fold, and the stimulation of biodegradation seen here is consistent with this increase.

Several things are noteworthy. The first is that the biodegradation of dispersed oil in these experiments is only marginally faster than it was in water collected at the same site under winter conditions, an apparent "half-life" of about 1 week at 21 °C (Fig. 2) compared with 11 days at 8 °C (Prince et al. 2013), which compares with 28 days (at 100 ppm oil) at 27.5 °C off the Penang, Malaysia shore (Zahed et al. 2011). The second is that these experiments used fresh seawater, not an acclimated inoculum (Venosa and Holder 2007; Campo et al. 2013), thus allowing the indigenous microbes to respond to the oil with the indigenous levels of biological nitrogen, phosphorus, and iron, etc. Next is the observation that the water under the floating slick in the undispersed experiments contained no detectable hydrocarbons (detection limit of the order of a few parts per billion for individual compounds), indicating that any hydrocarbons that dissolved out of the floating slick were promptly degraded by the indigenous microbes.

Our experiments offer the potential for developing a protocol for assessing the biodegradability of dispersed oil that might reveal differences between the biodegradability of dispersions generated by different dispersants. The tests described in the Introduction already offer a way of comparing the dispersion effectiveness of different commercial and experimental products. Building on our protocol, especially to assess and ensure its precision, might allow the development of tests for assessing whether particular formulations stimulate or transiently inhibit biodegradation (Varadaraj et al. 1995). As the protocol is developed, it will be important to ensure that the stirring is well controlled, since it is well known that mixing energy is a primary determinant of the differences seen in the current tests (Venosa et al. 2002; Clark et al. 2005). We use fresh seawater as inoculum because we do not have a clear idea of how to preserve an inoculum for repeated use. This may introduce variability, but attempts to maintain or preserve cultures also have their difficulties, most notably prolonged lag phases before biodegradation begins (Venosa and Holder 2007). More work is needed to determine the better option.

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To: Barron, Mace[Barron.Mace@epa.gov]; Craig Watts[craig@hydrosphere.net]; Holder, Edith[holder.edith@epa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov]
Cc: Peter Meyer[pmeyer@hydrosphere.net]; Cris Griffin[cgriffin@hydrosphere.net]
From: Craig Watts
Sent: Thur 9/15/2016 9:09:06 PM
Subject: RE: Final report for recent round of toxicity tests

Mace,

I will get started on those changes.

As I mentioned in the previous email, the report for Finasol should go out this same time next week.

Craig

Sent from my phone

-------- Original message --------
From: "Barron, Mace" <Barron.Mace@epa.gov>
Date: 9/15/16 4:19 PM (GMT-05:00)
To: Craig Watts <craig@hydrosphere.net>, "Holder, Edith" <holder.edith@epa.gov>, "Conmy, Robyn" <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>, Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Hey guys:

Just very few minor revisions requested from my technical review:

Table 2: A. Punctulata

* Acute column: please either spell out not applicable in the cell or add a footnote defining "NA".

*chronic column: replace NA with a footnote or something specifying the organism age or life stage tested.

Table 12:

*report the NOEC and IC24 values in uL/L

Please do provide a revised copy, as well as a revised excel sheet with the toxicity summary tables.

Thanks again for your work with EPA and Pegasus.

PS: also, could you update us what is next on your schedule for this work (e.g., finquel? Anything else to be completed from testing samples we have provided?

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Thursday, September 15, 2016 2:35 PM
To: Barron, Mace <Barron.Mace@epa.gov>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Mace,

Spreadsheets? You have a beautiful report in front of you!

Here is your spreadsheet.

Craig

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: Thursday, September 15, 2016 2:56 PM
To: Craig Watts <craig@hydrosphere.net>; Holder, Edith <holder.edith@epa.gov>; Conmy,

Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: RE: Final report for recent round of toxicity tests

Thank you!

I was able to download a copy and will provide a technical review in next few days.

Could you also provide a copy of just the tox results in excel format similar to what you provided for the dilbits (attached).

Much appreciated,

Mace

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Thursday, September 15, 2016 1:49 PM
To: Barron, Mace <Barron.Mace@epa.gov>; Holder, Edith <holder.edith@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>; Cris Griffin <cgriffin@hydrosphere.net>
Subject: Final report for recent round of toxicity tests

To all,

So much for our effort to simply and streamline the reports. The Corexit report weighs in at over 15 MB and 111 pages. Instead of choking everyone's email server, I will share a link to the file on our DropBox account:

https://www.dropbox.com/s/aoi238renwts50v/16119.pdf?dl=0

Please look over the report and let us know if you have any questions or if you would like to see any changes.

We have all of the testing completed for the Finasol product with the exception of the two acute EC50 tests; they are going up today. The report for Finasol should go out this same time next week.

Regards,

Craig

Providing Environmental & Product Toxicity Testing since 1986

Craig Watts, Lab Director

Hydrosphere Research

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Cc: Peter Meyer[pmeyer@hydrosphere.net]; Cris Griffin[cgriffin@hydrosphere.net]
From: Craig Watts
Sent: Thur 9/15/2016 7:34:30 PM
Subject: RE: Final report for recent round of toxicity tests
Toxicity Results.xlsx

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Craig

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Cc: Peter Meyer[pmeyer@hydrosphere.net]; Cris Griffin[cgriffin@hydrosphere.net]
From: Barron, Mace
Sent: Thur 9/15/2016 6:56:08 PM
Subject: RE: Final report for recent round of toxicity tests
15123 Tox Results.xlsx

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https://www.dropbox.com/s/aoi238renwts50v/16119.pdf?dl=0

Please look over the report and let us know if you have any questions or if you would like to see any changes.

We have all of the testing completed for the Finasol product with the exception of the two acute EC50 tests; they are going up today. The report for Finasol should go out this same time next week.

Regards,

Craig

Hydrosphere research

Providing Environmental & Product Toxicity Testing since 1986

Craig Watts, Lab Director

Hydrosphere Research

11842 Research Circle

Alachua, FL 32615-6817

T (386) 462-7889

www.hydrosphere.net

To: Conmy, Robyn[Conmy.Robyn@epa.gov]
From: Barron, Mace
Sent: Wed 10/26/2016 3:18:03 PM
Subject: RE: need 200 mL minimum of dispersant for the lab...

Wow, nice! Great fall color?

From: Conmy, Robyn
Sent: Wednesday, October 26, 2016 10:14 AM
To: Barron, Mace <Barron.Mace@epa.gov>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Thanks! Ha!!!! BOSC is all set. I already 'stole' some figures from one of your toxicity presentations ….. but of course your name is listed on the slide as the tech expert for all things tox in SHC 3.62 ☺

I'm in Victoria BC this week and loving life. It will be hard to leave the ocean, mountains, weather, stable government. Sigh.

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace
Sent: Wednesday, October 26, 2016 11:09 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Thank you!

Good luck with BOSC review. Need anything from me?

Mace

From: Conmy, Robyn
Sent: Wednesday, October 26, 2016 9:59 AM
To: Barron, Mace <Barron.Mace@epa.gov>
Subject: FW: need 200 mL minimum of dispersant for the lab...

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conmy.robyn@epa.gov

From: Craig Watts [mailto:craig@hydrosphere.net]
Sent: Wednesday, October 26, 2016 9:50 AM
To: Grosser, Robert <Grosser.Robert@epa.gov>; Conmy, Robyn <Conmy.Robyn@epa.gov>
Cc: Sundaravadivelu, Devi <sundaravadivelu.devi@epa.gov>; Peter Meyer <pmeyer@hydrosphere.net>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Bob,

Thank you. We will keep an eye out for it.

Regards,

Craig

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To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Craig Watts <craig@hydrosphere.net>
Cc: Sundaravadivelu, Devi <sundaravadivelu.devi@epa.gov>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Craig:

The Accell dispersant will be shipped today. Since you said you did not need it ASAP, it is coming by ground shipping. It should arrive in 3-4 business days.

Let me know if you need anything else.

Thanks.

Bob

Robert Grosser PhD

Environmental Microbiologist

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On-Site Contractor for the US EPA

26 West Martin Luther King Drive

Mail Location 190

Cincinnati, OH 45268

(513) 569-7529 office

(513) 569-7620 fax

From: Conmy, Robyn
Sent: Tuesday, October 25, 2016 6:24 PM
To: Grosser, Robert <Grosser.Robert@epa.gov>; devi.sundaravadivelu@ptsied.com
Subject: FW: need 200 mL minimum of dispersant for the lab...

As per the email from last week, please arrange for 200 ml of accel to be sent to hydrosphere.
Thanks

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

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conmy.robyn@epa.gov

From: Barron, Mace
Sent: Monday, October 24, 2016 5:06 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Holder, Edith <holder.edith@epa.gov>
Subject: need 200 mL minimum of dispersant for the lab...

Just fyi on what they used of corexit and finasol.

Please send new dispersant asap!

Thank you,

Mace

From: Peter Meyer [mailto:pmeyer@hydrosphere.net]
Sent: Monday, October 24, 2016 3:41 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Craig Watts <craig@hydrosphere.net>
Subject: RE: quick question...

Mace,

We ended up using about 160 mLs of each. So if I had to guess…

1) Corexit: We have about 1,200 mLs left over and…

2) Finasol: Probably about 700 mLs left over.

Neither container was full when we received them.

~Peter

From: Craig Watts
Sent: Saturday, October 22, 2016 7:22 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>
Subject: RE: quick question...

Mace,

I am going to have to hand this one off to Peter.

Craig

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To: Craig Watts <craig@hydrosphere.net>
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Thanks!

To: Conmy, Robyn[Conmy.Robyn@epa.gov]
From: Barron, Mace
Sent: Wed 10/26/2016 3:08:50 PM
Subject: RE: need 200 mL minimum of dispersant for the lab...

Thank you!

Good luck with BOSC review. Need anything from me?

Mace

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Sent: Wednesday, October 26, 2016 9:59 AM
To: Barron, Mace <Barron.Mace@epa.gov>
Subject: FW: need 200 mL minimum of dispersant for the lab...

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Thanks.

Bob

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Environmental Microbiologist

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Cc: Craig Watts <craig@hydrosphere.net>
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~Peter

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To: Grosser, Robert[Grosser.Robert@epa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov]
Cc: Sundaravadivelu, Devi[sundaravadivelu.devi@epa.gov]
From: Craig Watts
Sent: Wed 10/26/2016 1:57:00 PM
Subject: RE: need 200 mL minimum of dispersant for the lab...

Bob,

Peter just pointed out that the 200mL was based on how much we used last time. If we have to repeat any of the tests for whatever reason (dissolved oxygen crashes or we miss bracketing the LC50) we may need more. Is it possible to get 400 ml?

Regards,

Craig

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Do you recall how much we sent you? And how much you got left over?

We are getting ready to send a new dispersant to you and need to know the amount to send.

Thanks!

To: Conmy, Robyn[Conmy.Robyn@epa.gov]; Holder, Edith[holder.edith@epa.gov]
From: Bryan, Elisha
Sent: Thur 3/5/2015 1:37:02 PM
Subject: Re: List on shared drive

Edie and Robyn,

All right I will start contacting all of them except Finasol and those that I could not reach/asked for agreements with the SWAs.

The Dispersit SPC 1000 has an unlimited shelf life, but I can request a new sample if you prefer.

Thanks,

Elisha

From: Conmy, Robyn
Sent: Wednesday, March 4, 2015 1:55 PM
To: Holder, Edith
Cc: Bryan, Elisha
Subject: RE: List on shared drive

I made a slight change to the first paragraph. Otherwise, it looks great.

Thanks,

Robyn

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Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

727-692-5333 (mobile)

conmy.robyn@epa.gov

From: Holder, Edith
Sent: Tuesday, March 03, 2015 3:29 PM
To: Conmy, Robyn
Cc: Bryan, Elisha
Subject: FW: List on shared drive

Robyn and Elisha,

Thank you Elisha for writing this. It looks pretty good. One question is the first line

The U.S. Environmental Protection Agency is developing

Should that be past tense "has developed" since the protocol has been released for comments. Or is it correct in the present tense as the reason we want to obtain the dispersants is to set the decision rules?

Edie

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

From: Bryan, Elisha
Sent: Tuesday, March 03, 2015 9:10 AM
To: Holder, Edith
Subject: Re: List on shared drive

Hi Edie,

Here is the sample letter, any suggestions before Robyn looks it over?

I tried to make it as easy to understand as possible. I think it will still raise concerns with some of the manufacturers like with what happened with the SWAs.

Elisha

From: Holder, Edith
Sent: Monday, March 2, 2015 2:41 PM
To: Bryan, Elisha
Cc: Conmy, Robyn
Subject: RE: List on shared drive

Elisha,

We received a liter of Finasol OSR 52 within the last year, so we probably don't need that one.

We have close to a L of Dispersit SPC 1000, but it is approaching 10 years old. We have Corexit 9500 from 2010 (approx. 50 mL). There is approx. 20 mL of old JD2000. Then there are small amounts (<2 mL) of Sea Brat, ZI400, Nokomis 3F4, and Saf-ron Gold.

I would say contact everyone except the manufacturers of Finasol, but Robyn can weigh in with her opinion. Robyn should have the opportunity to edit the product request email prior to sending.

Linda Whiteley (MARINE D-BLUE CLEAN™) called me a couple of weeks ago asking about our SWA results. After telling her that we had no results ready for release, I told her that we would be doing more dispersant testing and that I would like to include her product in our testing. So perhaps the note to her could mention that.

Edie

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

From: Bryan, Elisha
Sent: Friday, February 27, 2015 4:51 PM
To: Holder, Edith
Subject: List on shared drive

Hi Edie,

I made the list and put it on the shared drive:

L:\Public\NRMRL-PUB\Holder\OilSpill

6 of the 19 listed are companies that we have previously tried to contact about SWA and either wanted us to sign an agreement or never responded. Another one has not gotten updated contact information and the company is in Japan. I highlighted these, maybe we already have them in the lab. Most of these do not seem to have a shelf life, do we still want to get new stuff if we already have it?

I can work on the email draft next week.

Have a nice weekend!

Elisha

Interspill 2015
Amsterdam, 24-26 March, 2015, Poster Presentation

Biodegradation of polycyclic aromatic hydrocarbons in Alaska North Slope crude oil: Effect of temperature and dispersant application

Mobing Zhuang[1], Pablo Campo-Moreno[1], Makram Suidan [2]*,
Albert D. Venosa (retired)[3], and Robyn Conmy[3]

1. Deparment of Biomedical, Chemical and Environmental Engineering, University of Cincinnati, 2901 Woodside Drive, Cincinnati, OH 45221, USA

2. Faculty of Engineering and Architecture, American University of Beirut, Bechtel Engineering Bldg. - 3rd flr. - Room 308 P.O. Box: 11-0236 Riad El Solh 1107 2020, Beirut, Lebanon

3. U.S. Environmental Protection Agency, National Risk Management Risk Laboratory, 26 W. MLK Drive Cincinnati, OH, 45268, USA

Abstract. Polycyclic aromatic hydrocarbons (PAHs), constituted of more than one fused benzene rings, are widely distributed contaminants and rise environmental and health concerns because of their toxic, mutagenic, and carcinogenic properties. A variety of microorganisms have been reported as capable of degrading PAHs, either under aerobic or anaerobic conditions. In this study, we conducted biodegradation experiment on Alaska North Slope (ANS) crude oil with two cultures isolated from the Gulf of Mexico and enriched in the laboratory. Cultures meso and cryo were originally collected by EPA's Gulf Ecology Division from surface and deepsea respectively, and then enriched in their laboratory at 25 °C (meso) and 5 °C (cryo). Naphthalene, fluorene, phenanthrene, dibenzothiophene, naphthobenzothiophene, pyrene, and chrysene along with the corresponding homologues are the most abundant PAHs found in ANS. Their biodegradability at 25 °C and 5 °C, with and without the application of dispersant C9500 and JD-2000 will be presented at the conference. Estimations of biodegradation rates and extents of PAHs are essential for oil spill response modeling and decision making.

Keywords: Polycyclic aromatic hydrocarbons, JD-2000, biodegradation, crude oil, surfactant, oil spills

1. Introduction

One of the primary means for the energy companies to expand their oil reserves is to drill deeper in the sea [1], although there has always been public concern over oil exploration offshore. In 2010, the explosion and sinking of an ultra-deepwater drilling rig called the Deepwater Horizon (DWH) resulted in the release of an unprecedented volume of oil in the deep sea, which was estimated to be 4.9 million barrels of light crude oil [2]. Only about 4-5% of the oil was removed by mechanical equipment during the DWH incident [2]. The response team made the early decision to apply dispersants on a large scale never before tried at the wellhead in the deep sea. Dispersants were also applied to the surface slick to prevent oil from reaching

* Corresponding author: Tel.: + 00961-1-347952; fax: + 00961-1-744462.
E-mail address: msuidan@aub.edu.lb

shorelines. The application of dispersant, especially at deepsea, was controversial due to the large exposure to the ecosystem and environmental safety concerns [3-6].

The use of dispersants can affect oil biodegradation in several ways. Early dispersant products were toxic to microorganisms, thus decreasing the extent of biodegradation or prolonging the lag period [7]. Dispersant could decrease the oil-water interfacial tension, thus breaking the oil slick to small droplets, the resulting small droplets increase the surface area for microbes to interact with. With a larger specific surface area, dissolution of oil chemicals is enhanced (according to Fick's First Law of Diffusion), which promotes microbial uptake and consumption [8, 9]. Thus, sufficient dispersion of oil could assist following biodegradation. We conducted biodegradation tests of chemical dispersions prepared with Alaska North Slope crude oil (ANS) and JD-2000, listed on National Contingency Product Plan Schedule (NCPPS), was reported to have satisfactory dispersant effectiveness and lower toxicity [10-12].

2. Experimental Setup

Three treatments, namely, oil alone, dispersed oil, and dispersant alone were studied to understand the biodegradability of oil and dispersant separately and in mixture. Table 1 presents the experimental layout of the tests conducted for all the oils at 5 and 25 °C. The table also includes sampling events. Triplicate killed controls (KCs) containing 500 mg/L of sodium azide were included to account for possible abiotic losses. After preparation, the flasks were placed on orbital shakers operated at 200 rpm and kept at the corresponding temperatures 5 or 25 °C. At a given sampling event, triplicate flasks of each treatment were sacrificed.

Table 1. Summary of Experimental Layout.

Test	Temperature	Treatment	Sampling Events	Sample Replicates	Total Experimental Units (EU)
ANS-JD-2000	5 °C	ANS dispersed by JD-2000	11	3	33
		ANS alone	11	3	33
		Killed ANS control	1	3	3
		Killed ANS+JD-2000 control	11	3	33
Sampling Events: days 0, 2, 4, 8, 12, 16, 24, 32, 40, 48, 56					
ANS-JD-2000	25 °C	ANS dispersed by JD-2000	9	3	27
		ANS alone	9	3	27
		Killed ANS control	1	3	3
		Killed ANS+JD-2000control	9	3	27
Sampling Events: days 0, 2, 4, 8, 12, 16, 24, 32, 40					
				Total EUs	**186**

The samples containing oil were extracted, concentrated, and analyzed for oil components by GC/MS on a model 6890 gas chromatograph coupled with a model 5973 mass spectrometer from Agilent (Palo Alto, CA). The analyzed aromatics (PAHs) included 2-, 3- and 4- ring groups with both the parent compounds and alkylated homologues [C_{0-4} naphthalenes (nap), C_{0-3} -dibenzothiophenes (dbt), C_{0-3} -fluorenes (flu), C_{0-4} -napthbenzothiophenes (nbt), C_{0-4} -phenanthrenes/anthracenes (phe), C_{0-2} -pyrenes (pyr), and C_{0-4} -

chrysenes (cry)]. The concentrations of all these analytes were normalized to the corresponding 17α(H), 21β(H)-hopane values [13]. The DB-5 MS column (30 m × 0.25 mm I.D., 0.25 μm film thickness) used in GC/MS was from J&W (Palo Alto, CA).

3. Results

3.1 25 °C Experiment.

In both dispersed ANS and ANS alone treatments, the biodegradation of PAHs at 25 °C followed similar patterns (Fig. 1 A –B, open symbols). The overall concentration dropped moderately from 0 to 2 d before sharply decreasing between days 2 to 4. The extent of removal at day 2 was higher in the treatment with JD-2000 (20% vs. 9%). The first-order rates (presented in Table 2) of C_{0-4}-NAP, C_{0-1}-PHE, C_{0-1}-FLU, and C_{0-1}-DBT were marginally higher (less than 1.5 times) in the dispersed ANS samples than in ANS alone treatment. Nevertheless, the enhancement reached more than 2 fold for C_2-PHE, C_2-FLU, and C_2-DBT.

Fig. 1: Biodegradation of hopane-normalized total PAHs at both temperatures without (A) and with (B) JD-2000 dispersant in the ANS experiment.

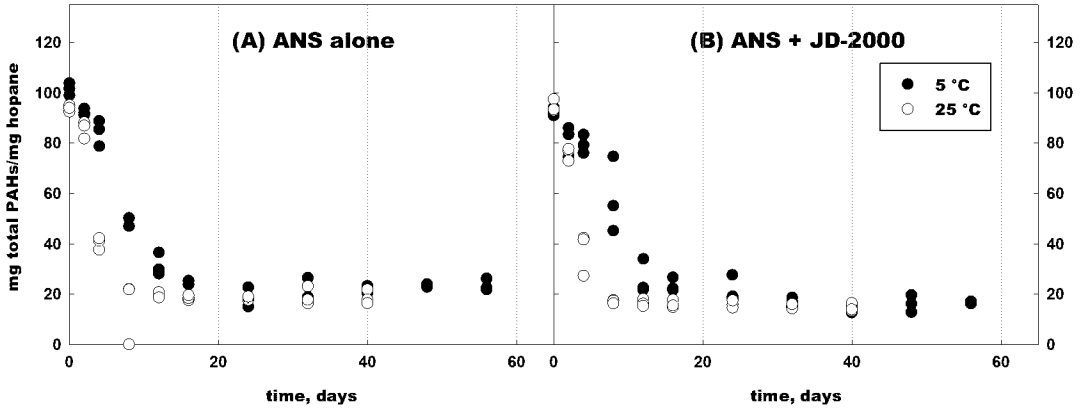
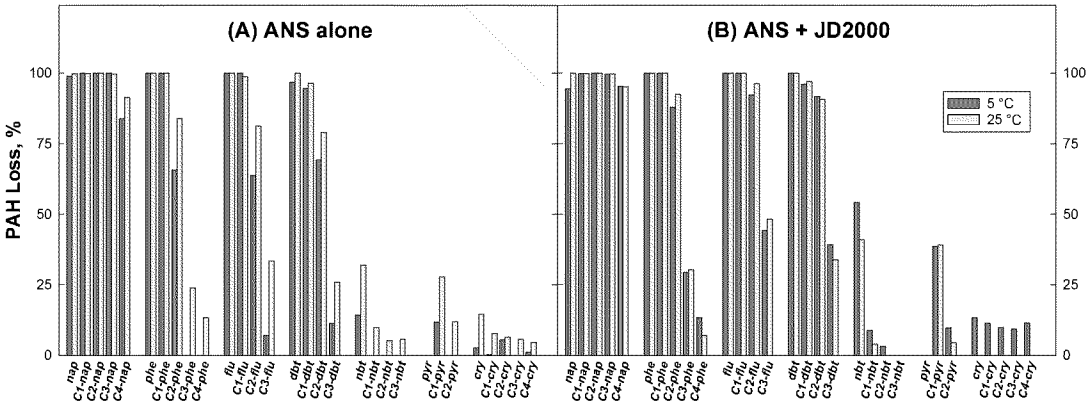


Fig. 2: Loss percentage of individual PAH in the absence (A) and presence (B) of JD-2000 at 5 °C and 25 °C.



3.2 5 °C Experiment.

At the low temperature, the biodegradation of PAHs in the ANS – JD-2000 experiment was slow from 0 to 4 d, regardless of the presence of dispersant (Fig. 1 A-B, closed symbols). The hopane-normalized time series of the total PAH concentrations in the JD-2000 dispersed ANS treatment did not differ from ANS alone. The first-order rates of individual PAHs, presented in Table 2, exhibited negligible differences between those two treatments, with the exception of naphthalene, which degraded faster in the absence of JD-2000, i.e.,-0.44 d^{-1} vs. -0.27 d^{-1}).

Table 2. First-order degradation rate coefficients and standard deviations (sd) of individual PAHs

Compound	5 °C ANS alone		25 °C ANS alone		5 °C ANS+JD2000		25 °C ANS+JD2000	
	rate (d-1)	sd (d-1)	rate (d-1)	sd (d-1)	rate (d-1)	sd (d-1)	rate (d-1)	sd (d-1)
nap	0.44	0.04	0.34	0.06	0.27	0.02	0.38	0.05
C1-nap	0.23	0.02	0.37	0.06	0.22	0.02	0.47	0.04
C2-nap	0.17	0.02	0.33	0.06	0.16	0.02	0.37	0.05
C3-nap	0.27	0.02	0.27	0.04	0.2	0.03	0.3	0.04
C4-nap	0.13	0.02	0.16	0.02	0.11	0.02	0.2	0.02
phe	0.19	0.01	0.32	0.06	0.17	0.02	0.33	0.05
C1-phe	0.12	0.01	0.2	0.03	0.11	0.02	0.24	0.03
C2-phe	0.05	0.01	0.07	0.01	0.07	0.01	0.15	0.02
C3-phe	0	0	0	0	0	0	0	0
C4-phe	0	0	0	0	0	0	0	0
flu	0.19	0.02	0.3	0.05	0.15	0.02	0.33	0.04
C1-flu	0.15	0.02	0.18	0.02	0.12	0.02	0.24	0.03
C2-flu	0.05	0.01	0.06	0.01	0.14	0.03	0.16	0.02
C3-flu	0	0	0	0	0	0	0	0
dbt	0.25	0.01	0.31	0.06	0.24	0.02	0.34	0.05
C1-dbt	0.13	0.01	0.2	0.04	0.12	0.01	0.26	0.03
C2-dbt	0.04	0.01	0.06	0.01	0.06	0.01	0.15	0.02
C3-dbt	0	0	0	0	0	0	0	0

* Naphthobenzothiophene , Pyrene, and Chrysene Homologues are not listed in this table because were persisted during the experiment (rate coefficient equals 0).

4. Discussion and Conclusion

To summarize, JD-2000 had certain improvement on the biodegradation of aromatics in ANS at 25 °C, while the impact was less significant at 5 °C. PAHs comprising two or three fused rings have a certain solubility in water (e.g., naphthalene: 30 mg/L), and their uptake and biodegradation occurs mostly within the aqueous phase [14, 15]. Surfactants could increase the transfer rate of these more soluble compounds from the oil to aqueous phase by forming small oil droplets, which increase the interfacial area.

Additionally, it was observed that the %loss of C_4-NAP, $C_{2\text{-}4}$-PHE, $C_{2\text{-}3}$-FLU, C_3-DBT were higher in the presence of JD-2000, regardless of temperature, as depicted in the right-hand panels (dispersed ANS treatments) of Figure 2. Chemical dispersion increased the extent of removal of these less soluble PAH

compounds, which could be critical in terms of the aromatics toxicity issue [16, 17].

5. Acknowledgements

We thank Jan Kurtz and Diane Yates from EPA's Gulf Ecology Division (GED) at Gulf Breeze, FL, who collected the water samples in the GOM and performed the enrichments and provided them for our experiments. The research was a product of the U.S. Environmental Protection Agency's National Risk Management Research Laboratory (NRMRL) and was funded by EPA, NRMRL, Cincinnati, OH, under Pegasus Technical Services, Inc. Contract EP-C-11-006.

6. References

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To: Conmy, Robyn[Conmy.Robyn@epa.gov]
Cc: Bryan, Elisha[Bryan.Elisha@epa.gov]
From: Holder, Edith
Sent: Tue 3/3/2015 8:29:28 PM
Subject: FW: List on shared drive
sample letter.docx

Robyn and Elisha,

Thank you Elisha for writing this. It looks pretty good. One question is the first line

The U.S. Environmental Protection Agency is developing

Should that be past tense "has developed" since the protocol has been released for comments. Or is it correct in the present tense as the reason we want to obtain the dispersants is to set the decision rules?

Edie

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

From: Bryan, Elisha
Sent: Tuesday, March 03, 2015 9:10 AM
To: Holder, Edith
Subject: Re: List on shared drive

Hi Edie,

Here is the sample letter, any suggestions before Robyn looks it over?

I tried to make it as easy to understand as possible. I think it will still raise concerns with some of the manufacturers like with what happened with the SWAs.

Elisha

From: Holder, Edith
Sent: Monday, March 2, 2015 2:41 PM
To: Bryan, Elisha
Cc: Conmy, Robyn
Subject: RE: List on shared drive

Elisha,

We received a liter of Finasol OSR 52 within the last year, so we probably don't need that one.

We have close to a L of Dispersit SPC 1000, but it is approaching 10 years old. We have Corexit 9500 from 2010 (approx. 50 mL). There is approx. 20 mL of old JD2000. Then there are small amounts (<2 mL) of Sea Brat, ZI400, Nokomis 3F4, and Saf-ron Gold.

I would say contact everyone except the manufacturers of Finasol, but Robyn can weigh in with her opinion. Robyn should have the opportunity to edit the product request email prior to sending.

Linda Whiteley (MARINE D-BLUE CLEAN™) called me a couple of weeks ago asking about our SWA results. After telling her that we had no results ready for release, I told her that we would be doing more dispersant testing and that I would like to include her product in our testing. So

perhaps the note to her could mention that.

Edie

Edith Holder

Pegasus Technical Services, Inc.

On-Site Contractor to the U.S. EPA

ORD/NRMRL/LRPCD

26 W. Martin Luther King Dr.

Cincinnati, OH 45268

Phone: 513-569-7178

Email: holder.edith@epa.gov

From: Bryan, Elisha
Sent: Friday, February 27, 2015 4:51 PM
To: Holder, Edith
Subject: List on shared drive

Hi Edie,

I made the list and put it on the shared drive:

L:\Public\NRMRL-PUB\Holder\OilSpill

6 of the 19 listed are companies that we have previously tried to contact about SWA and either wanted us to sign an agreement or never responded. Another one has not gotten updated contact information and the company is in Japan. I highlighted these, maybe

we already have them in the lab. Most of these do not seem to have a shelf life, do we still want to get new stuff if we already have it?

I can work on the email draft next week.

Have a nice weekend!

Elisha

Dear Dispersant Manufacturer,

The U.S. Environmental Protection Agency is developing a revision to the Dispersant Efficacy for inclusion in the Federal Register, 40 CFR Appendix C to Part 300 Subpart J. I am a contractor with EPA's Office of Research and Development, National Risk Management Research Laboratory (NRMRL), Land Remediation and Pollution Control Division (LRPCD), working on this research.

As your product is listed on the U.S. EPA National Contingency Plan Product Schedule, we would like to include your product in our research and are interested in procuring a small quantity. I am contacting all manufacturers listed so that we can take into consideration the different characteristics of the dispersants.

This research will not change the current status of your product on the Product Schedule. More information on the proposed revisions and who to contact for comments can be found here: https://www.federalregister.gov/articles/2015/01/22/2015-00544/national-oil-and-hazardous-substances-pollution-contingency-plan#h-44.

Could you please send me a price quote for one liter / quart of *DISPERSANT*?

Thank you,

Elisha Bryan

Pegasus Technical Services, Inc.
On-Site Contractor to the U.S. EPA
ORD/NRMRL/LRPCD
26 W. Martin Luther King Dr.
Cincinnati, OH 45268
Phone: 513-569-7047
Email: bryan.elisha@epa.gov

To: Grosser, Robert[Grosser.Robert@epa.gov]; Conmy, Robyn[Conmy.Robyn@epa.gov]
Cc: Sundaravadivelu, Devi[sundaravadivelu.devi@epa.gov]; Peter Meyer[pmeyer@hydrosphere.net]
From: Craig Watts
Sent: Wed 10/26/2016 1:50:13 PM
Subject: RE: need 200 mL minimum of dispersant for the lab...

Bob,

Thank you. We will keep an eye out for it.

Regards,

Craig

Hydrosphere research

Providing Environmental & Product Toxicity Testing since 1986

Craig Watts, Lab Director

Hydrosphere Research

11842 Research Circle

Alachua, FL 32615-6817

T (386) 462-7889

www.hydrosphere.net

From: Grosser, Robert [mailto:Grosser.Robert@epa.gov]
Sent: Wednesday, October 26, 2016 9:28 AM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Craig Watts <craig@hydrosphere.net>
Cc: Sundaravadivelu, Devi <sundaravadivelu.devi@epa.gov>
Subject: RE: need 200 mL minimum of dispersant for the lab...

Craig:

The Accell dispersant will be shipped today. Since you said you did not need it ASAP, it is coming by ground shipping. It should arrive in 3-4 business days.

Let me know if you need anything else.

Thanks.

Bob

Robert Grosser PhD

Environmental Microbiologist

Pegasus Technical Services, Inc.

On-Site Contractor for the US EPA

26 West Martin Luther King Drive

Mail Location 190

Cincinnati, OH 45268

(513) 569-7529 office

(513) 569-7620 fax

From: Conmy, Robyn
Sent: Tuesday, October 25, 2016 6:24 PM
To: Grosser, Robert <Grosser.Robert@epa.gov>; devi.sundaravadivelu@ptsied.com
Subject: FW: need 200 mL minimum of dispersant for the lab...

As per the email from last week, please arrange for 200 ml of accel to be sent to hydrosphere. Thanks

<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>/<>

Robyn N. Conmy, Ph.D.

Research Ecologist

USEPA/NRMRL/LRPCD

26 West MLK Drive

Cincinnati, Ohio 45268

513-569-7090 (office)

513-431-1970 (EPA mobile)

727-692-5333 (Personal mobile)

conmy.robyn@epa.gov

From: Barron, Mace

Sent: Monday, October 24, 2016 5:06 PM
To: Conmy, Robyn <Conmy.Robyn@epa.gov>; Holder, Edith <holder.edith@epa.gov>
Subject: need 200 mL minimum of dispersant for the lab...

Just fyi on what they used of corexit and finasol.

Please send new dispersant asap!

Thank you,

Mace

From: Peter Meyer [mailto:pmeyer@hydrosphere.net]
Sent: Monday, October 24, 2016 3:41 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Craig Watts <craig@hydrosphere.net>
Subject: RE: quick question...

Mace,

We ended up using about 160 mLs of each. So if I had to guess…

1) Corexit: We have about 1,200 mLs left over and…

2) Finasol: Probably about 700 mLs left over.

Neither container was full when we received them.

~Peter

From: Craig Watts
Sent: Saturday, October 22, 2016 7:22 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Peter Meyer <pmeyer@hydrosphere.net>
Subject: RE: quick question...

Mace,

I am going to have to hand this one off to Peter.

Craig

From: Barron, Mace [mailto:Barron.Mace@epa.gov]
Sent: Friday, October 21, 2016 11:20 AM
To: Craig Watts <craig@hydrosphere.net>

Subject: quick question...

How much dispersant (e.g., corexit) did you guys use in the tox tests?

Do you recall how much we sent you? And how much you got left over?

We are getting ready to send a new dispersant to you and need to know the amount to send.

Thanks!

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Cc: Sundaravadivelu, Devi[sundaravadivelu.devi@epa.gov]
From: Grosser, Robert
Sent: Wed 10/26/2016 1:27:37 PM
Subject: RE: need 200 mL minimum of dispersant for the lab...

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The Accell dispersant will be shipped today. Since you said you did not need it ASAP, it is coming by ground shipping. It should arrive in 3-4 business days.

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Robert Grosser PhD

Environmental Microbiologist

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Thanks!

To: Conmy, Robyn[Conmy.Robyn@epa.gov]; Sundaravadivelu, Devi[sundaravadivelu.devi@epa.gov]
From: Grosser, Robert
Sent: Wed 10/26/2016 10:59:48 AM
Subject: RE: need 200 mL minimum of dispersant for the lab...

We will take care of this Robyn.

Bob

From: Conmy, Robyn
Sent: Tuesday, October 25, 2016 6:24 PM
To: Grosser, Robert <Grosser.Robert@epa.gov>; devi.sundaravadivelu@ptsied.com
Subject: FW: need 200 mL minimum of dispersant for the lab...

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Thanks!

Dispersant effectiveness under high salinity conditions following a subsurface jet release of oil

B. Robinson[1], T. King[1], R. Conmy[2], M. Boufadel[3], S. Ryan[1], C. McIntyre[1], P. Toole[1]

[1] Bedford Institute of Oceanography, Dept. Fisheries and Oceans Canada
[2] U.S. Environmental Protection Agency, Office of Research and Development, NRMRL
[3] New Jersey Institute of Technology

International Oil Spill Conference 2017
May 15-18, 2017
Long Beach, California
Submitted abstract on 8/24/16

The potential for oil and gas exploration and production activities in close proximity to hypersaline water bodies, such as brine channels in the Arctic or brine pools in the Gulf of Mexico, raises the need to evaluate dispersant effectiveness (DE) at high salinities. To investigate the effect of hypersaline conditions on DE, a series of experiments were conducted using an underwater jet release of oil in a flume tank located at the Bedford Institute of Oceanography in Nova Scotia, Canada. The 32 m long flume tank has a seawater capacity of 30,000 L and is equipped with a high flow pumping system that generates horizontal water currents of up to 5 cm/s. A subsurface oil injection system is used to create a jet release of oil that travels horizontally down the length of the tank. Experiments were conducted using two hydrocarbon products (Alaska North Slope crude oil and Intermediate Fuel Oil 120) at four different salinities (28, 50, 75, 100 ppt). Each experiment was conducted with two jet releases, one injection with untreated oil and a second injection with oil and dispersant (Corexit 9500A) at a dispersant-to-oil ratio (DOR) of 1:25. Results will be presented from the insitu droplet size analysis using a LISST particle size analyzer that was used to determine how increased salinity affected the droplet size distribution (DSD). A suite of hydrocarbon fluorometers was also used to monitor the movement of the dispersed oil, and these findings will be summarized along results from the analysis of discrete water samples collected at various locations and time points for the determination of DE.

Poster Abstract

Casey O'Laughlin, Brent A. Law, Vanessa S. Zions, Thomas L. King, Brian Robinson, Yongsheng Wu. *The size versus settling relations of oil-mineral aggregates derived from diluted bitumen.* International Oil Spill Conference, May 15-18 2017, Long Beach, California.

A series of experiments to elucidate size versus settling velocity relationships of chemically- and physically-dispersed OMAs (oil-mineral aggregates) derived from diluted bitumen have been performed. Experiments are completed in an enclosed wave tank facility, and are designed to characterize warm water (>10 °C), high-sediment concentration (~50 mg/L) conditions in the presence and absence of chemical dispersant (Corexit®EC-9500A). Previous wave tank experiments operated at a low sediment concentration (~10 mg/L), and with a short 2-hour settling period, failed to produce OMAs. In these more recent experiments with higher sediment concentration, the settling phase is extended to 24 hours. The resulting occurrence of OMAs in wave tank experiment water is confirmed via microscope. Machine-vision floc cameras were used to produce high-resolution images of settling particles covering a size range from 45 microns to 1 millimeter, at image capture rates of up to 11 frames per second. These images are used to determine the settling velocity of individual particles. Size-settling relationships derived from similar imagery collected during previous lab experiments using lab-created OMAs show a broad range of settling velocities of OMA particles (0.04-23.9 mm/s). Analysis of images from recent 24 hour wave tank experiments is ongoing, and it is expected that a similarly broad range of size versus settling relationships will result from 'naturally' formed OMAs in these experiments.

To: Conmy, Robyn[Conmy.Robyn@epa.gov]; Holder, Edith[holder.edith@epa.gov]
From: Barron, Mace
Sent: Mon 10/24/2016 9:05:45 PM
Subject: need 200 mL minimum of dispersant for the lab...

Just fyi on what they used of corexit and finasol.

Please send new dispersant asap!

Thank you,

Mace

From: Peter Meyer [mailto:pmeyer@hydrosphere.net]
Sent: Monday, October 24, 2016 3:41 PM
To: Barron, Mace <Barron.Mace@epa.gov>
Cc: Craig Watts <craig@hydrosphere.net>
Subject: RE: quick question...

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B. Robinson[1], T. King[1], R. Conmy[2], M. Boufadel[3], S. Ryan[1], C. McIntyre[1], P. Toole[1]

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Long Beach, California
Submitted abstract on 8/24/16

The potential for oil and gas exploration and production activities in close proximity to hypersaline water bodies, such as brine channels in the Arctic or brine pools in the Gulf of Mexico, raises the need to evaluate dispersant effectiveness (DE) at high salinities. To investigate the effect of hypersaline conditions on DE, a series of experiments were conducted using an underwater jet release of oil in a flume tank located at the Bedford Institute of Oceanography in Nova Scotia, Canada. The 32 m long flume tank has a seawater capacity of 30,000 L and is equipped with a high flow pumping system that generates horizontal water currents of up to 5 cm/s. A subsurface oil injection system is used to create a jet release of oil that travels horizontally down the length of the tank. Experiments were conducted using two hydrocarbon products (Alaska North Slope crude oil and Intermediate Fuel Oil 120) at four different salinities (28, 50, 75, 100 ppt). Each experiment was conducted with two jet releases, one injection with untreated oil and a second injection with oil and dispersant (Corexit 9500A) at a dispersant-to-oil ratio (DOR) of 1:25. Results will be presented from the insitu droplet size analysis using a LISST particle size analyzer that was used to determine how increased salinity affected the droplet size distribution (DSD). A suite of hydrocarbon fluorometers was also used to monitor the movement of the dispersed oil, and these findings will be summarized along results from the analysis of discrete water samples collected at various locations and time points for the determination of DE.